1. Introduction

In the Central Valley of California the Sacramento River and San Joaquin River converge to form the Sacramento-San Joaquin Delta before flowing into San Francisco Bay (Figure 1). The Sacramento River serves as a catchment for waters draining the entire northern portion of the Central Valley and drains approximately 70,000 km^2. The San Joaquin River drains approximately 35,055 km^2 of the southern portion of the Central Valley. Omernik (1987) designated the Sacramento River and San Joaquin River contiguous basins as the Central Valley ecoregion. This ecoregion is characterized by irrigation-subsidized agriculture and water development activities have significantly modified stream flow regimes. All large rivers and most small streams are dammed for flood control and runoff storage. Stored water is transported through natural channels or constructed canals for irrigation of agricultural lands, municipal and industrial needs and to fulfill environmental requirements. Annual precipitation at various geographical areas within the Sacramento River basin averages 36 to 63 cm. In the northern and southern portions of the San Joaquin River basin annual precipitation averages 38 and 13 cm, respectively. This rainfall occurs primarily in the November through February period. The predominant landscape feature of the Sacramento River and San Joaquin River basins is agriculture (Domagalski *et al*., 1998; Groneberg *et al*., 1998). These activities and modifications in the Central Valley have resulted in widespread alteration of riparian zones, waterway geomorphology, flow and water quality, raising concerns about the health of the region's aquatic ecosystems.

Agriculture-dominated waterways (ADWs) receive greater than fifty percent of flow from irrigation runoff. Irrigation occurs primarily during the dry season (March through October). ADWs can be natural streams, constructed waterways, or a combination of both. There are over 9,173 and 8,400 km of natural and constructed ADWs in the Sacramento River and San Joaquin River watersheds, respectively; natural ADWs constitute approximately 10 percent of the total waterbodies in the two watersheds. A wide range of physical, chemical and biological conditions exists in both natural and constructed agricultural drains.

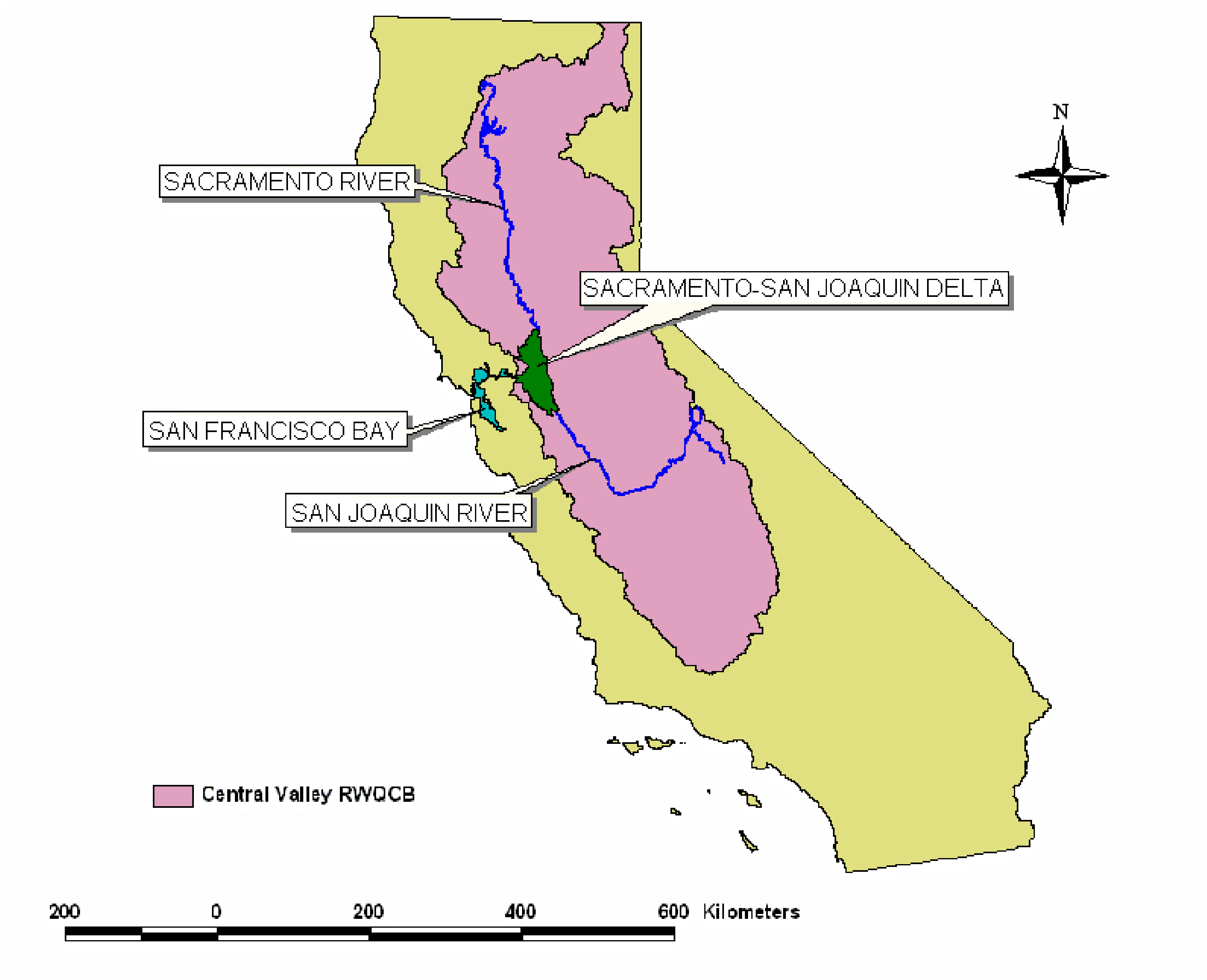

Figure 1. California's Central Valley, Sacramento and San Joaquin watersheds, and Delta.

Due to the seasonality of rain and snowmelt many waterways in the Sacramento River and San Joaquin River watersheds are intermittent unless supplemented by irrigation water. In fact, many waterways within the Central Valley are dominated either by water that will be used for irrigation or by irrigation runoff (ISWP, 1991). The agriculture-dominated segments of most waterways usually occur in the lower valley floor (< 165 m elevation).

Many publications and reports document that runoff from agricultural lands degrade surface water quality in California (e.g., see review article of de Vlaming *et al*., 2000 and also Foe and Connor, 1989, 1991; Finlayson *et al*., 1991; Norberg-King *et al.*, 1991; Foe and Sherpline, 1993; Foe, 1995; Kuivila and Foe, 1995; MacCoy *et al.*, 1995; Deanovic *et al.* 1996, 1998; Domagalski, 1996; Ross *et al.*, 1996; Domagalski *et al.*, 1998; Kratzer, 1997; de Vlaming *et al.*, 1998 Dubrovsky *et al.*, 1998; Foe *et al.*, 1998; Werner *et al*., 2000; Larsen *et al.*, 1998a, b; Panshin *et al.*, 1998; Hunt *et al.*, 1999, 2003; Anderson *et al.*, 2002, 2003a, b; de Vlaming, 2002; Holmes and de Vlaming, 2003; Phillips *et al*., 2004; de Vlaming *et al*., 2004a, b). Pesticides (including herbicides, insecticides, fungicides) totaling millions of kilograms are applied annually in Sacramento River basin (CDPR, 2002). Much of the toxicity to aquatic species in ADWs has been linked to insecticides (e.g., de Vlaming *et al*., 2000, 2004). In response to recent changes in the California Water Code and in recognition of these findings, the Central Valley Regional Water Quality Control Board (CVRWQCB) has re-evaluated and updated its regulatory program for runoff (discharges) from irrigated agricultural lands, primarily irrigation return flows (surface runoff and subsurface drainage) and storm water runoff. Since 1982 irrigated agriculture in the Central Valley had been conditionally waived from waste discharge requirements if the following conditions were met: (1) For irrigation return water, the discharger had to minimize sediment to meet Basin Plan (Water Quality Control Plan) turbidity objectives and had to prevent concentrations of materials toxic to fish or wildlife, and (2) For storm water runoff, a waiver was allowed when no water quality problems were contemplated and no federal National Pollutant Discharge Elimination System (NPDES) permit was required (CVRWQCB Resolution No. 82-036). These waiver conditions were in place when the contract supporting this study was written and one goal of this project was to help the CVRWQCB assess water quality in the agricultural drains. In July 2003 the CVRWQCB adopted new waiver conditions that apply to discharges from irrigated lands that are significantly more stringent and require monitoring to verify compliance with water quality objectives.

The purpose of this study was to gain a more complete understanding of the relationship between water quality in agricultural drains and irrigation runoff. The State Water Resources

Control Board (SWRCB)/Central Valley Regional Water Quality Control Board (CVRWQCB) contracted with the University of California, Davis Aquatic Toxicology Laboratory (UCD ATL) to conduct this investigation. The objectives of this pilot project included: (1) Evaluation of water quality, primarily through the use of aquatic species toxicity testing, in a limited number of agricultural drains in the San Joaquin River and Sacramento River watersheds, (2) Identification of the causes (e.g., sediment, contaminants, salt, etc.) of any water quality impacts, (3) Determination of the sources of contaminants based on the identified causes of impairments, (4) Conduct a literature review related to potential impacts of agricultural runoff on water quality and aquatic biota, and (5) Use the data and information gained in this investigation as a basis for recommendations regarding future monitoring and assessment of agricultural runoff.

2. Materials and Methods

2.1 Sample Sites and Schedule

The primary criteria for site selection were: (1) Drainage dominated by agricultural irrigation return flow during months without rainfall, (2) Land use patterns surrounding the site predominated by mixed row and field crops (except for two sites where the primary land use is rice culture) and (3) Site is at a location near where the drainage water is discharged into a stream or river. Because this was a pilot project intended to examine water quality in irrigation return water, there was no intent to select sites representative and inclusive of all agricultural drainage throughout the Central Valley. Nor was there intent to select equal numbers of sites in the counties of the Central Valley. Funding level limited the total number of sites that could be investigated. Thus, the intent was to investigate fewer sites more intensely. Dispersing sites widely throughout the Central Valley would have required multiple field crews and considerable time in the field. To conserve funds for actual testing, sites were clustered in counties relatively near UCD ATL.

Table 1 lists and Figures 2 and 3 illustrate the sampling sites in the Sacramento River and San Joaquin River watersheds, respectively. Maps of the individual sites are provided in Volume II, Appendix A. Sampling dates are summarized in Table 2. Samples were collected from 11 sites within the Delta and San Joaquin River watershed and 13 sites in the Sacramento River watershed. The project employed a fixed sampling schedule in which each site was sampled approximately every three weeks (beginning in March) for *C. dubia* and *P. promelas* toxicity tests. In addition, *C. dubia* was tested in a 'special study' conducted on 11 June 2003 following an aerial pesticide application in Colusa and Yolo Counties. When toxicity was observed in a sample collected during the fixed sampling events or in the special study, that site was re-sampled within 48 hours. To estimate the duration of toxicity at that site, the increased frequency of sampling continued until no toxicity was observed in samples from that site. The significance and ecological relevance of toxicity at a site are related to duration, magnitude and frequency of that toxicity.

Table 1. Summary of GPS coordinates for individual sites.

Site #	Site Description	Latitude	Longitude
1[A]	8 Mile & Rio Blanco Rds.	38.0505	-121.41753
2[B]	Unnamed Slough @ Woodsbro Rd. & Burns Cutoff Levee	37.94174	-121.36912
3	Return Irrigation Drain @ MCD Rd.	37.96983	-121.46227
4	SJR Source Water to Canal	37.99402	-121.42045
5	Drain @ Wing Levee Rd.	37.85659	-121.37801
6[C]	Tom Paine Sl. @ El Rancho Rd.	37.76898	-121.37445
7	Lone Tree Creek @ Newcastle Rd.	37.8622	-121.21009
8	Little John Creek @ Newcastle Rd.	37.8763	-121.21068
9	Walthal Slough @ Woodward Ave.	37.77046	-121.29227
10	Westport Drain @ Jennings Rd.	37.53674	-121.06676
11	Unnamed Drain @ Pomelo Rd.	37.46904	-121.06274
12a	Drain @ Robben Rd.	38.41628	-121.78608
12b	Drain @ Robben Rd. & Midway Rd.	38.38011	-121.78632
13	Drain @ Ulatis Creek @ Hwy. 113	38.33838	-121.8233
14[D]	Creek @ Hawkins Rd.	38.35865	-121.84846
15	Lateral to Gordon Slough @ Rd. 19	38.71881	-121.95438
16	Gordon Slough @ Rd. 19	38.71465	-121.92439
17[E]	Drain @ Mace Blvd.	38.5116	-121.69517
18	Stone Corral Creek @ 4 Mile Rd.	39.29337	-122.11665
19	East Drain @ 4 Mile Rd.	39.30535	-122.11652
20[F]	West Drainage @ Del Paso Rd.	38.6563	-121.56059
21	Sand Creek @ Miller Rd.	39.056779	-122.02279
22[G]	Sycamore Slough @ Hwy. 45	38.88107	-121.84364
23	Knight's Landing Ridge Cut South @ Rd. 16	38.74842	-121.69489
24	Knight's Landing Ridge Cut North @ Rd. 16	38.74894	-121.69498

[A]: After 7/3/03, [B]: After 6/12/03, [C]: After 7/24/03, [D]: After 6/17/03, [E]: After 6/5/03, [F]: After 8/5/03, [G]: After 6/11/03

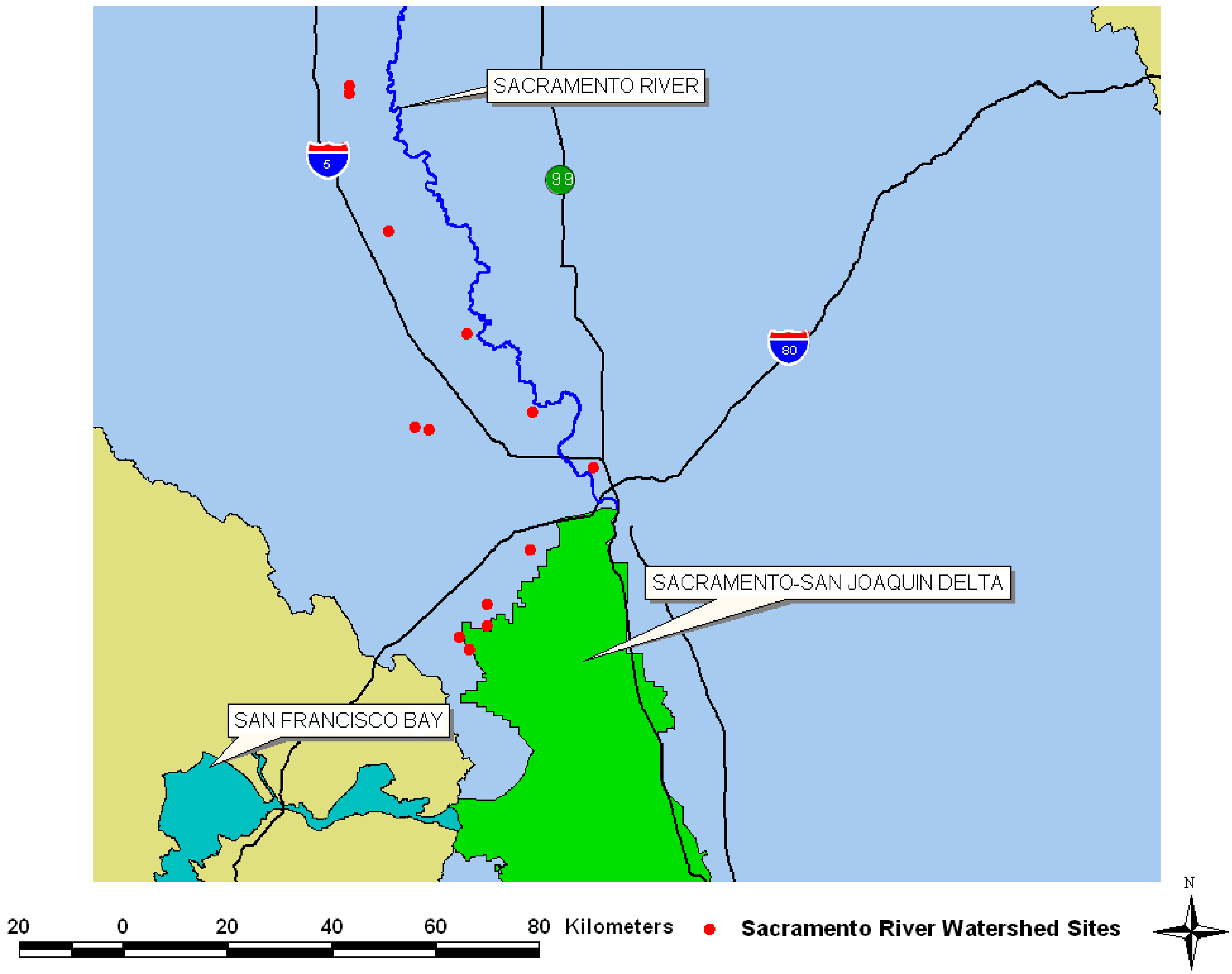

Figure 2. Diagrammatic representation of sampling site locations in the Sacramento River watershed and Delta.

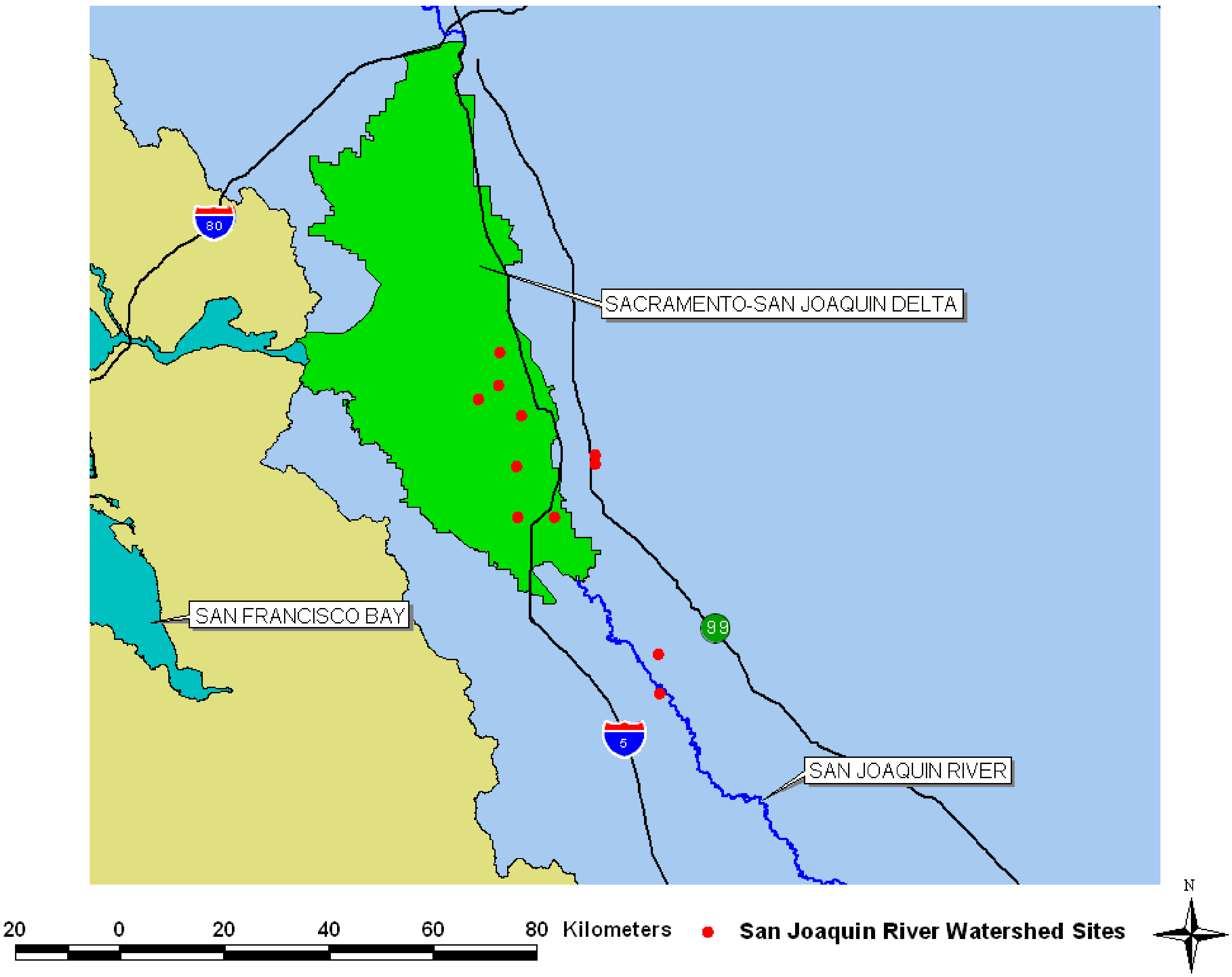

Figure 3. Diagrammatic representation of sampling site locations in the San Joaquin River watershed and Delta.

Table 2. Summary of sample dates of preplanned, special study and follow-up events from 26 March 2003 to 7 October 2003.** (Duplicate site numbers are explained in the text.)

		Round				
Site	Site Description	1	2	S.S.[1]	S.S.a[2]	3
1	Beaver Sl. @ Blossom Rd.	4/3/03	5/29/03			6/12/03
1	8 Mile & Rio Blanco Rd.					
2	Unnamed Sl. @ Woodsbro Rd.	4/15/03	5/27/03			
2	Unnamed Sl. @ Woodsbro Rd. & Burns Cutoff Levee					6/12/03
3	Return Irrigation Drain @ McDonald Rd.	4/3/03	5/29/03			6/12/03
4	SJR Source Water to Canal	4/1/03	5/27/03			6/12/03
5	Drain @ Wing Levee Rd.	3/26/03	5/27/03			6/12/03
6	Drain @ Bowman Rd.	4/1/03	5/27/03			6/12/03
6	Tom Paine Sl. @ El Rancho Rd.					
7	Lone Tree Creek @ Newcastle Rd.	3/26/03	5/22/03			6/10/03
8	Little John Creek @ Newcastle Rd.	4/1/03	5/22/03			
9	Walthal Sl. @ Woodward Ave.	4/1/03	5/27/03			6/10/03
10	Westport Drain @ Jennings Rd.	3/26/03	5/22/03			6/10/03
11	Unnamed Drain @ Pomelo Rd.	3/26/03	5/22/03			6/10/03
12	Drain @ Midway Rd. East of Pedrick Rd.		5/29/03			
12a	Drain @ Robben Rd.					6/17/03
12b	Drain @ Robben Rd. & Midway Rd.					6/17/03
13	Drain @ Ulatis Creek @ Hwy. 113	4/3/03	5/29/03			6/17/03
14	Drain @ Midway Rd. West of Schroeder	4/3/03	5/29/03			
14	Creek @ Hawkins Rd.					6/17/03
15	Lateral to Gordon Sl. @ Rd. 19	4/8/03	6/3/03			6/19/03
16	Gordon Sl. @ Rd. 19	4/8/03	6/3/03			6/19/03
17	Willow Sl. @ Rd. 27	4/8/03				
17	Drain @ Mace Blvd.		6/5/03	6/11/03		6/19/03
18	Stone Corral Creek @ 4 Mile Rd.	4/10/03	6/5/03	6/11/03	6/16/03	6/24/03
19	East Drain @ 4 Mile Rd.	4/10/03	6/5/03	6/11/03	6/16/03	6/24/03
20	Elk Creek @ Hahn & Miller's Rd.	*	*	*	*	*
20	West Drainage @ Del Paso Rd.					
21	Sand Creek @ Miller Rd.	4/10/03	6/5/03	6/11/03		6/24/03
22	Drain South of Rd. 14		6/5/03			
22	Sycamore Slough @ Hwy. 45			6/11/03	6/16/03	6/24/03
23	Knight's Landing Ridge CT South @ Rd. 16		6/3/03			6/19/03
24	Knight's Landing Ridge CT North @ Rd. 16	4/8/03	6/3/03			6/19/03

[1]: Special Study

[2]: Rounds with letters indicate follow up to samples exhibiting toxicity.

[3]: Resampled for cerio reset up, not toxicity.

* Not sampled due to low flow or dryness.

** Table continued on following page.

Table 2, continued.

Number										
4	5	5a	5b	6	6a	7	7a	7b	7c	8
7/3/03	7/24/03			8/14/03		9/4/03				9/25/03
7/3/03	7/24/03			8/14/03		9/4/03				9/25/03
7/3/03	7/24/03			8/14/03		9/4/03				9/25/03
7/3/03	7/24/03			8/14/03		9/4/03				9/25/03
7/3/03	7/24/03			8/14/03		9/4/03				9/25/03
7/3/03	7/24/03			8/14/03		9/4/03				9/25/03
7/1/03	7/22/03			8/12/03		9/2/03				9/23/03
7/1/03	7/22/03			8/12/03		9/2/03	9/4/03			
7/1/03	7/22/03			8/12/03		9/2/03				9/23/03
7/1/03	7/22/03	7/25/03	7/29/03	8/12/03		9/2/03				9/23/03
7/1/03	7/22/03	7/25/03[3]		8/12/03		9/2/03				9/23/03
7/8/03	7/29/03			8/19/03		9/9/03	9/12/03	9/15/03	9/19/03	9/30/03
7/8/03	7/29/03			8/19/03		9/9/03				9/30/03
7/8/03	7/29/03			8/19/03		9/9/03				9/30/03
7/8/03	7/29/03			8/19/03		9/9/03				9/30/03
7/10/03	7/31/03			8/21/03	8/25/03	9/11/03				10/2/03
7/10/03	7/31/03			8/21/03		9/11/03				10/2/03
7/10/03	7/31/03			8/21/03		9/11/03				
7/15/03	8/5/03			8/26/03		9/16/03				10/7/03
7/15/03	8/5/03			8/26/03		9/16/03				10/7/03
*										
	8/5/03			8/26/03		9/16/03				10/7/03
7/15/03	8/5/03			8/26/03		9/16/03				10/7/03
7/15/03	8/5/03			8/26/03		9/16/03				10/7/03
7/10/03	7/31/03			8/21/03		9/11/03				10/2/03
7/10/03	7/31/03			8/21/03		9/11/03				10/2/03

2.2 Sample Collection and Storage

UCD ATL collected two hundred thirty-four samples between March and October 2003. Samples were collected as subsurface grabs from mid-channel (whenever possible) in pre-cleaned, 1-gallon, amber glass bottles. One additional liter was collected in high density polyethylene containers for turbidity analysis. Field measurements of pH, specific conductance (SC), dissolved oxygen (DO) and temperature were recorded for each site. Field measurements were compared to laboratory measurements to ensure consistency of water quality parameters after sample storage. Immediately after collection samples were placed in an ice chest on wet ice for transport to the UCD ATL where they were stored in the dark at 4 ± 2ºC. All samples were employed in toxicity tests within 48 hours of sample collection.

2.3 Toxicity Testing

Ceriodaphnia dubia (a cladoceran zooplankton species) and larval *Pimephales promelas* (a cyprinid minnow) toxicity testing procedures followed those outlined in Methods for Measuring Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (US EPA, 2002) with some exceptions. These are static-renewal tests with mortality as the only endpoint/response determined. Aspects of these procedures that differ from the US EPA methods and the rationale for using them are outlined below.

While US EPA methods do not specifically recommend aeration of the renewal water, the UCD ATL protocols include aeration. This deviation is employed because the ambient samples tested at UCD ATL frequently require aeration to prevent oxygen super-saturation. Aeration time is limited until samples come to 102% saturation to minimize the loss of volatile toxicants.

2.3.1 *Ceriodaphnia dubia*

The *C. dubia* used in these tests were from UCD ATL cultures. The cladocerans were cultured in Sierra Springs™ water amended to US EPA (2002) moderately hard specifications. The *C.*

dubia assay consisted of four replicate glass vials. The US EPA recommends using plastic cups for the *C. dubia* toxicity test. However, plastic adsorbs organic compounds so plastic cups are not appropriate for determining the role of organic compounds in *C. dubia* toxicity. Each vial contained 18ml of sample and five *C. dubia* each. Less than 24-hour-old *C. dubia,* all born within a 20-hour period, were employed at test initiation. *C. dubia* were transferred into a vial containing *Selenastrum*, YCT (a mixture of yeast, organic alfalfa and trout chow) and 18 ml of fresh sample water daily. The test was incubated in a temperature-controlled room kept at 25 ± 2°C with a 16:8 hour light:dark photoperiod for four days. Mortality was measured daily and upon test termination.

2.3.2 *Pimephales promelas*

The minnows were obtained from Aquatox, (Hot Springs, Arkansas). The *P. promelas* assay consisted of four replicate 600ml beakers, each containing 250ml of sample and 10 larval fathead minnows. Minnows were less than 48-hours-old at test initiation. Fish were fed twice daily with brine shrimp, *Artemia* nauplii. Approximately 80% of test solution was renewed daily. Dead fish, *Artemia* and debris were removed from the test beakers daily. The test solution was incubated in a water bath at 25 ± 2°C under ambient laboratory light with a 16:8 hour light:dark photoperiod for seven days. Mortality was measured daily upon test solution renewal and test termination.

2.3.3 Quality Assurance

US EPA test acceptability for *C. dubia* and larval *P. promelas* 96-hour tests requires 90% or greater survival in the controls. When the control performance did not meet test acceptability criteria, all data from the test were rejected. Each toxicity test survey included a laboratory control. The laboratory control waters varied for each species. For the *C. dubia* assay, the laboratory control was Sierra Springs™ water amended to a hardness of 80 to 100 mg/L as $CaCO_3$ (SSEPAMH). De-ionized water amended to a hardness of 80 to 100 mg/L as $CaCO_3$ (DIEPAMH) was used the as the control water in the larval fish assay. A positive control, reference-toxicant test was performed monthly for each species using NaCl. These tests included the laboratory control and a dilution series of NaCl in laboratory control water. The purpose of these tests was to assess any deviations in organism sensitivity (i.e., response) to a known toxicant. The LC/EC_{50} for each reference toxicant test was plotted to ascertain whether it fell within the acceptable range relative to previous results. If test results did not fall within acceptable ranges, results of concurrent toxicity tests were deemed suspect. The method the UCD ATL uses to calculate the acceptable range of variation differs somewhat from that recommended by the US EPA. The US EPA recommends that acceptable data fall within two standard deviations of the mean for the total data set. The UCD ATL accepts data that fall within two standard deviations of the running mean. These standard deviations, at any one point on the control chart, represent the standard deviation for that particular data point and nineteen previous points. The UCD ATL uses reference toxicant data to track changes in animal sensitivity/responsiveness over time.

Measures were taken to ascertain test repeatability (precision) at UCD ATL. Precision was assessed by including ambient sample blind duplicates and toxicant spikes into laboratory control water. Matrix (i.e., ambient water from the Sacramento and San Joaquin River watersheds) spikes were performed to assess matrix effects on test organism response to a known toxicant and to evaluate precision. Laboratory control water trip blanks were tested to appraise whether transport affected toxicity. These test/laboratory performance measures were applied to approximately ten percent of all samples. Ambient water duplicates were collected using the same procedures as for the primary samples, but were labeled with a different identification number so laboratory technicians could not recognize duplicates. Equivalent responses were expected from organisms in the primary sample and its duplicate. The matrix spike and matrix spike duplicate were prepared in the laboratory from a randomly chosen site sample. The laboratory spike was laboratory control water amended with the same toxicant as the matrix spike and matrix spike duplicate. Duplicates were compared by statistical analysis. If statistical differences ($p<0.05$) were observed between duplicates, data were considered suspect.

2.3.4 Water Quality

Water quality parameters of temperature, pH, dissolved oxygen (DO) and electrical conductivity (EC) were measured on all test samples upon initiation of the test. DO and pH were recorded on 24-hour-old samples immediately before test sample renewal. Measurements were taken with a Check Temp™ digital thermometer, pH was measured with a Beckman 255 pH meter, DO was measured with a YSI model 58 oxygen meter with a 5700 series probe and EC was measured with a YSI model 30 EC meter. All meters were calibrated daily according to the manufacturers' instructions. Ammonia was measured using the Aquaquant® ammonium kit within 24 hours of sample receipt. Hardness and alkalinity were measured on all samples utilizing titrimetric methods within 24 hours of sample receipt. Total suspended solids (TSS) and/or Suspended Solid Concentrations (SSC) were measured using ASTM D 3977-97 (1997) methods within ten days of sampling.

2.3.5 Statistical Analysis

Toxicity was defined as a statistically significant difference ($p<0.05$) in test species mortality between an ambient sample and the laboratory control water. *C. dubia* and larval fish mortality data were analyzed for normality with the Shapiro-Wilks Test and for homogeneity of variance with Bartlett's Test. When data fit normal distributions and manifested homogeneous variances, they were analyzed by Analysis of Variance (ANOVA) followed by Dunnett's mean separation tests. If data deviated significantly from normality or had heterogeneous variances, they were log transformed to improve the data distribution. ANOVA and Dunnett's mean separation tests were used to analyze data that was successfully transformed. If log transformation did not establish normality or homogeneity of variance, the nonparametric Bonferroni corrected Wilcoxan Rank Sum tests were performed to compare ambient sample data to the control. These statistical analyses differ from those outlined in US EPA (2002). US EPA protocols were designed for whole effluent toxicity testing in which effluent samples are tested in a dilution series. The statistical analyses recommended by US EPA (2002) were designed to analyze data from a dilution series. The approach taken during this study was to assess water quality at a particular site compared to laboratory control water as well as to other sites (conservative approach). No dilution series were performed during initial screening of samples. As a result, the US EPA (2002) statistical protocols were not appropriate for the data obtained during this study. UCD ATL staff consulted the UCD Statistics Laboratory (Neil Willits) to determine the most appropriate statistical analyses for these data. The statistician approved the analyses described above.

2.4 Toxicity Identification Evaluations (TIEs)

Information on toxicity of ambient samples is more useful if the causes are known. Thus, a primary objective in this study was to identify the cause(s) of toxicity in toxic samples through the application of TIEs. TIEs consist of physical, chemical and toxicological manipulations designed to identify the specific toxicant(s) responsible for toxicity.

2.4.1 Dilution Series

Dilution series tests were performed to determine the magnitude/potency of toxicity in toxic samples. Results of these tests were used to estimate the toxic units (TUs) in a toxic sample. Toxic units were estimated by dividing the 100% sample by the lowest sample dilution causing toxicity. For example, if the sample diluted to 25% causes toxicity, the sample consists of at least four TUs of toxic substance(s). With this approach the TU estimate accuracy depends on the number of dilutions in the series (more accuracy with more dilutions). TUs contributed by individual toxic chemicals can also be estimated from the analytical chemistry results. In this context, a TU is defined as the concentration of a specific chemical present in a toxic sample divided by the 96-hour LC_{50} concentration for the species of interest. An LC_{50} is defined as the concentration of a chemical that causes 50% mortality in 96 hours. This approach tends to be more robust and accurate than the dilution series estimate. Toxic units can be added when multiple toxicants are present (assuming that the individual toxic compounds act additively). The more equivalent the two estimates, the more conclusive the results are from the TIE. Toxic units contributed by individual toxicants can be compared to toxic units determined by dilution of the ambient water sample. Dilution series tests were performed on samples causing

100% mortality to *C. dubia* within 48-hours. Dilutions consisted of 100, 50, 25, 12.5 and 0% of the sample. Dilutions are made with laboratory control water.

2.4.2 Phase I TIEs

The purpose of Phase I TIEs is to identify the class(es) of contaminant(s) causing the toxicity. The toxicity tests associated with TIE procedures were performed as described above; additional sample manipulations were performed to reveal the cause(s) of toxicity. Solid phase extraction (SPE) columns remove nonpolar organic chemicals from aqueous test samples. Toxic samples were passed through an SPE column and this water sample was tested along with the unmanipulated toxic sample. Control water also was passed through a SPE column and served as one of the procedure controls. Chemicals absorbing to the column were eluted with methanol. This methanol eluate was added to control water and tested along with the method blank. If the toxicant is a nonpolar organic chemical, the ambient sample and control water amended with eluate exhibit equivalent mortality, while the sample passed through the SPE column results in reduced or no mortality. Disodium Ethylenediamine Tetraacetate (EDTA) and Sodium Thiosulfate (STS) form complexes with various heavy metals, rendering them unavailable to biota. Three concentrations of each EDTA and STS are added separately to toxic samples and tested along with the 'original' toxic sample and controls. If the toxicant is one of these heavy metals, the ambient sample exhibits mortality while the ambient sample amended with EDTA or STS results in reduced or no mortality.

Air stripping sometimes reduces or removes volatiles and/or ammonia from waters. Caution must be applied to interpretation of air stripping results because the procedure is not standardized or quantitative. Toxic samples were air stripped and tested along with the 'original' non-stripped sample and controls. If the toxicant is volatile, the ambient sample exhibits mortality, while the air stripped sample results in reduced or no mortality. In the *C. dubia* Phase I TIEs, samples are amended with piperonyl butoxide (PBO). PBO inhibits or reduces toxicity caused by metabolically activated organophosphorous (OP) insecticides such as diazinon, chlorpyrifos and malathion (Bailey *et al.*, 1996). 100 µg/L PBO was added to the toxic samples. The 'original' ambient test sample and the ambient test sample amended with PBO were tested along with the appropriate controls. If the toxicant is a metabolically activated OP insecticide, the ambient test sample exhibits *C. dubia* mortality while the ambient test sample amended with PBO results in reduced or no mortality.

2.4.3 Phase II TIEs

The purpose of Phase II TIEs is to identify the constituent(s) causing or contributing to the toxicity. If the Phase I TIE suggested that the toxicity was due to nonpolar organic constituents, the sample was concentrated on SPE columns and fractionated by eluting the column with 50, 70, 75, 80, 85, 90, 95 and 100% methanol. Each fraction was then spiked into control water and tested. This procedure serves to eliminate chemicals that do not contribute to mortality (such chemicals will be in non-toxic fractions). Chemical analyses are applied to identify constituents in the toxic fractions.

2.4.4 Chemical Analyses

As a component of TIE procedures, chemical analyses were conducted on toxic samples. Analyses were performed, under the supervision of Dr. Peter Green in the laboratory of Dr. Thomas Young in the Department of Civil & Environmental Engineering, UCD. Mass spectrometry was the primary means of identifying unknown toxicants; the exact approach used was dependent on the results of the Phase I TIE described above. If a metal was the suspected toxicant because toxicity was removed by adding a chelating agent, the original sample was analyzed by inductively coupled plasma mass spectrometry (ICP-MS). If a nonpolar organic chemical was the suspected toxic agent because toxicity was removed after passing the sample through an SPE cartridge, a solvent wash of the SPE was analyzed by gas chromatography/mass spectrometry (GC/MS). This approach also was followed if the Phase I TIE indicated that the suspected toxicant was a metabolically activated OP insecticide. If a volatile organic compound (VOC) appeared to be responsible because the toxicity was removed by air stripping, the sample was analyzed by purge and trap gas chromatography/mass spectrometry (PT-GC/MS). If toxicity was not removed by chelation, SPE, or air stripping the cause was presumed to be a polar organic compound and analyses were conducted using liquid chromatography-mass spectrometry (LC-MS).

Sub-samples of toxic samples collected at sites 8, 10, 12a and 15 (total of seven samples) were transferred to the Department of Fish and Game, Nimbus Laboratory and AQUA-Science, Davis CA for organophosphorus insecticide analysis by GC/MS and ELISA, respectively.

3. Results

A total of 234 samples were collected between March 26 and October 7, 2003. Twenty-eight of these samples were included in quality assurance (laboratory performance evaluation) determinations.

3.1 Toxicity Testing

One objective of this project was to evaluate water quality through the use of aquatic species toxicity testing. Tables summarizing multiple toxicity tests are included in this report to give an overview of the results. Detailed tables summarizing individual toxicity test results for each event are provided in Volume II, Appendix B and C.

US EPA (2002) requires that performance of each species in laboratory control water meet specific criteria for test data to be considered valid. All tests conducted in this project met those acceptability criteria. For both acute *C. dubia* and larval *P. promelas* tests, US EPA requires that 90% of organisms in the control water survive. Of the 81 *C. dubia* tests performed, all 81 met test acceptability criteria. All 42 of the larval fish tests also met test acceptability criteria.

3.1.1 *Ceriodaphnia dubia*

3.1.1.1 Preplanned Sampling Events

Sample collection dates at the twenty-five sites for the preplanned sampling events are presented in Appendix I, Table 1. *C. dubia* mortality in site samples from the preplanned sampling events is summarized in Appendix II, Table 1. Four samples (less than 2%) caused *C. dubia* mortality. These samples were from four sites (Little John Creek at Newcastle Rd.—Site 8, Westport Drain at Jennings Rd.—Site10, Drain at Robben Rd—Site 12a and Lateral to Gordon Slough—Site 15) collected during the regularly scheduled sampling events. No other samples collected at these sites during the preplanned sampling events were toxic to the cladoceran.

3.1.1.2 Special Study

Samples were collected from Site 17--Drain at Mace Blvd., Site 18--Stone Corral Creek at 4 Mile Rd., Site 19--East Drain at Four Mile Rd., Site 21--Sand Creek at Miller Rd. and Site 22--Sycamore Slough at Highway 45 and tested with *C. dubia*. Three (Sites 18, 19 and 22) of the five samples caused *C dubia* mortality (Appendix II, Table 2).

3.1.1.3 Follow-up on Toxic Samples

Another objective was to identify causes of test organism mortality. Test results are summarized in Appendix II, Table 3. A primary toxicant is considered to be the substance that causes a majority of the observed mortality. Full-blown Phase I Toxicity Identification Evaluation (TIE) procedures were conducted on six samples. When a Phase I TIE revealed that toxicity was not due to a metal or volatile chemical, Phase I TIEs conducted on follow-up samples from those sites eliminated related manipulations to minimize cost. Such abbreviated Phase I TIEs were conducted on three samples. Of the 203 samples investigated, only 10 (less than 5%) caused statistically significant *C. dubia* mortality. Follow-up performed on samples exhibiting statistically significant mortality are summarized below.

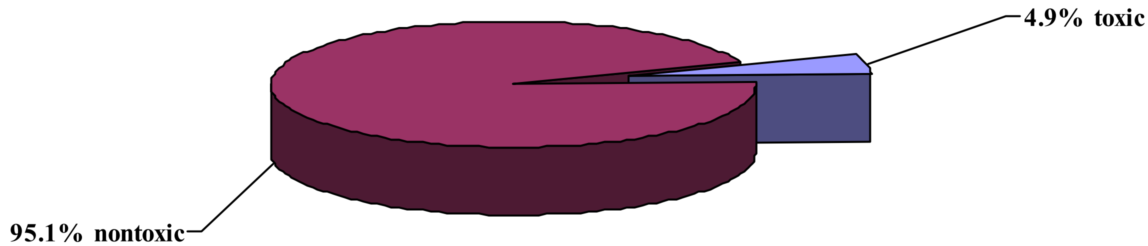

Figure 4. Percent of samples nontoxic and toxic to *C. dubia*.

In the March 26th Site 7 sample (Lone Tree Creek @ Newcastle Rd.) there was implication of low level, but not statistically significant, toxicity to *C. dubia*. *C. dubia* exhibited 40% mortality within 72 hours of test initiation. Consequent to the low level toxicity signal there was no follow-up on this sample.

3.1.1.3.1 Sycamore Slough at Highway 45 (Site 22)

C. dubia exhibited 100% mortality within 48 hours of test initiation in the Site 22 (Yolo Co.) sample collected on 11 June 2003. A dilution series test indicated a minimum of 1 toxic unit (TU) of toxicant(s) in this sample. A Phase I TIE was not performed on this sample due to a loss of toxicity during storage. The site was re-sampled on 16 June 2003. No statistically significant *C. dubia* mortality was noted in this sample.

3.1.1.3.2 Stone Corral Creek at Four Mile Road (Site 18)

The Site 18 (Colusa Co.) sample collected on 11 June 2003 resulted in 70% *C. dubia* mortality within 48 hours. In the Phase I TIE C8 solid phase extraction (SPE) removed toxic nonpolar organic chemical(s) from the sample. Add-back experiments (methanol elution of the SPE column added to control water) implicated a highly hydrophobic chemical(s) as a contributor to test species mortality. We were unable to identify this chemical(s). Phase I TIE procedures yielded no evidence of metal toxicity. On 16 June 2003 this site was re-sampled. The sample was not toxic to *C. dubia*.

US EPA TIE procedures have strengths and limitations. The procedures do require updates and improvements. The inability of ATL to specifically identify the hydrophobic chemical(s) causing/contributing to toxicity was not due to ATL mistakes, but rather that the US EPA procedures have limitations and are incomplete. In the US EPA TIE procedures, hydrophobic compounds (such as pyrethroid insecticides) present a particular problem for both the TIE procedures and chemical analytical procedures. Considerable refinement of TIE procedures is needed, especially as thousands of new chemicals come into the market every year. Procedures developed in the late 1980s are not completely effective in current times. Maintaining pace with the proliferation of chemicals is a definite challenge. Refinement and development of these procedures will be costly.

3.1.1.3.3 East Drain @ Four Mile Road (Site 19)

A sample collected on 11 June 2003 at Site 19 (Colusa Co.) elicited 50% *C. dubia* mortality within 96 hours. Piperonyl butoxide (PBO) reduced mortality demonstrating that a metabolically activated OP insecticide(s) was the primary contributor to mortality. Air stripping alleviated mortality in the sample, implying that a volatile toxicant(s) could be contributing to toxicity. However, consequent to experiments conducted during the course of this project (see below) we are not confident that the air stripping procedure reduces mortality of only volatile chemicals. The site was re-sampled on 16 June 2003. No acute toxicity to *C. dubia* was seen it this sample.

3.1.1.3.4 Westport Drain at Jennings Road (Site 10)

A sample collected on 22 July 2003 in Stanislaus County at Site 10 caused 100% *C. dubia* mortality within 24 hours. A dilution series test indicated approximately 5.5 TUs of toxicant(s) in this sample. Phase I TIE results linked mortality to a metabolically activated OP insecticide(s). In a Phase II TIE the toxicant eluted in the 75% and 80% methanol (MeOH), implicating chlorpyrifos as the primary toxicant (Kuivila and Crepeau, 1999; Bailey *et al*., 1996). Analyses performed by AQUAScience Davis, CA and California Department of Fish and Game Nimbus Lab documented 3.7-4.7 TUs of chlorpyrifos in this toxic sample. Chlorpyrifos TUs were based on sample concentrations determined by ELISA and GC/MS divided by the *C. dubia* 96-hour chlorpyrifos LC_{50} (78ng/L—based on many determinations at UCD ATL). Re-sampling of the site occurred 25 July 2003. Testing revealed 100% *C. dubia* mortality within 24 hours. A dilution series test signified approximately 5.8 TUs of toxicant(s) in this sample. Phase I TIE, ELISA and GC/MS results echoed those of the sample collected three days earlier. That is, chlorpyrifos (3.8-4.5 TUs) was the only or primary contaminant responsible for mortality. On 29 July 2003 the site was again re-sampled. That sample was not toxic to *C. dubia.* These data suggest that there was at least a three-day, high magnitude/concentration pulse of chlorpyrifos at this site.

3.1.1.3.5 Lateral to Gordon Slough at Road 19 (Site 15)

A sample collected at Site 19 (Yolo Co.) on 21 August 2003 resulted in100% *C. dubia* mortality within 24 hours. Approximately 4.6 TUs of toxicant were indicated by a dilution series test. A Phase I TIE indicated that mortality in this sample was consequent to a metabolically activated OP insecticide(s). Air stripping implied that a volatile chemical could be involved in the toxicity. Phase II TIE results revealed that toxicant eluted in the 75% and 80% MeOH fractions, implicating chlorpyrifos as the cause of mortality. GC/MS and ELISA analyses confirmed 2.4 TUs of chlorpyrifos in this sample. The site was re-sampled on 29 August 2003. Testing did not reveal statistically significant *C. dubia* mortality.

3.1.1.3.6 Little John Creek at New Castle Road (Site 8)

C. dubia exhibited 100% mortality within 48 hours in the Site 8 (San Joaquin Co.) sample collected 2 September 2003. Approximately 1.5 TUs of toxicant(s) were suggested in a dilution series test. A metabolically activated OP insecticide(s) was again linked to test species mortality by Phase I TIE procedures. Sample air stripping implied a possibility that a volatile chemical contributed to toxicity. The toxicant eluted in the 75% and 80% MeOH fractions of the Phase II TIE implicating chlorpyrifos as the primary cause of mortality. Chemical analysis documented 1.2 TUs of chlorpyrifos in the sample. Re-sampling at the site occurred on 4 September 2003. The *C. dubia* test indicated that the sample was not toxic.

3.1.1.3.7 Drain at Robben Road (Site 12a)

On 9 September 2003 a sample was gathered at Site 12a (Solano Co.) that evoked 100% *C. dubia* mortality within 24 hours. A dilution series test denoted approximately 2.7 toxicant(s) TUs in this sample. Phase I TIE procedures linked sample-caused mortality to a metabolically activated OP insecticide(s). Air stripping reduced sample mortality: evoking the possibility that a volatile chemical was involved. In the Phase II TIE the toxicant eluted in the 70%, 75% and 80% MeOH fractions implicating chlorpyrifos as the primary cause of test species mortality. Both GC/MS and ELISA analyses confirmed 2.4 TUs of chlorpyrifos in this sample.

On 12 September 2003 the site was sampled again. This sample evoked 100% *C. dubia* mortality within 24 hours. About 2.7 TUs of toxicant(s) were estimated by a dilution series test. Phase I TIE procedures in association with GC/MS and ELISA analyses identified chlorpyrifos and the primary toxicant, present at 2.6 TUs in the sample. The site was re-sampled on 15 September 2003; this sample resulted in 60% *C. dubia* mortality within 48 hours. This sample was estimated, with a dilution series test, to contain approximately 1 TU of toxicant(s). Re-sampling of the site occurred on 19 September 2003. The sample was not toxic to *C. dubia.* These data suggest that there was at least a six-day, high magnitude/concentration pulse of chlorpyrifos at this site.

3.1.1.4 Pesticide Use

Table 3 summarizes potential duration, estimated magnitude and cause(s) of toxicity detected during this project. Phase I and II TIEs, in association with chemical analyses, linked *C. dubia* mortality to chlorpyrifos in all toxic samples collected in July, August and September 2003. The finding of toxicity associated with chlorpyrifos is not new for Central Valley waters. Several other studies conducted at UCD ATL and at other laboratories have revealed that chlorpyrifos is a frequent contaminant in irrigation runoff during and after periods of application of this OP insecticide. Numerous water bodies have been listed as impaired by this chemical on the 2002 Clean Water Act Section 303(d) List of Water Quality Limited Segments as adopted by the State Water Resources Control Board and approved by the U.S. Environmental Protection Agency (http://www.swrcb.ca.gov/tmdl/303d_lists.html). As a result, the CVRWQCB is developing total maximum daily loads (TMDLs) for chlorpyrifos discharges to the San Joaquin River and the Sacramento-San Joaquin Delta, and has just adopted a Basin Plan amendment with a new control program addressing chlorpyrifos discharges to several creeks in the Sacramento urban area.

Chlorpyrifos use data for 2002 for each county where toxic samples were collected are presented in Table 4. These data are presented to illustrate the temporal pattern of chlorpyrifos use. 2003 data will be substituted when they become available. The major uses of chlorpyrifos in the six counties were on walnuts, alfalfa, almonds, structural pest control and wine grapes. Chlorpyrifos use (http://www.ipm.ucdavis.edu) was highest during March, June, July and August 2002 (Appendix III, Table 1).

Table 3. Summary of causes of toxicity to *C. dubia* for preplanned sampling events, the special study, and follow-up samples from 26 March 2003 to 7 October 2003.

Site #	Site Description	Sample Date	County	Duration[3]	Magnitude (TU[1])	Magnitude (TU[2])	Cause
8	Little John Creek at Newcastle Rd.	9/2/03	San Joaquin	Unknown	1.5	1.2	Chlorpyrifos
10	Westport Drain @ Jennings Rd.	7/22/03	Stanislaus	4+ Days	5.5	3.7 – 4.7	Chlorpyrifos
10	Westport Drain @ Jennings Rd.	7/25/03	Stanislaus		5.8	3.8 – 4.5	Chlorpyrifos
12a	Drain @ Robben Rd.	9/9/03	Solano	7+ Days	2.7	2.4	Chlorpyrifos
12a	Drain @ Robben Rd.	9/12/03	Solano		2.8	2.6	Chlorpyrifos
12a	Drain @ Robben Rd.	9/15/03	Solano		~1	0.6 – 1.2	Chlorpyrifos
15	Lateral to Gordon Slough @ Rd. 19	8/21/03	Yolo	Unknown	4.6	2.4	Chlorpyrifos
18	Stone Corral Creek @ 4 Mile Rd.	6/11/03	Colusa	Unknown	~1	NAV	Non-polar organic, possibly including hydrophobic compound(s)
19	East Drain @ 4 Mile Rd.	6/11/03	Colusa	Unknown	1.0	NAV	Non-polar organic, OP insecticide
22	Sycamore Slough @ Hwy. 45	6/11/03	Yolo	Unknown	~1	NAV	Non-polar organic, volatile/labile

[1]: An observed Toxic Unit (TU) is defined as the 100% percent sample divided by the percentage of sample that kills 50% of the organisms. The percentage of sample that kills 50% of the organisms is determined by the maximum likelihood-probit method.
[2]: An expected TU is defined as the concentration of a chemical in a water sample divided by the 96-hr test species LC_{50} (concentration causing 50% mortality within 96 hrs) for that chemical.
NAV: Data Not Available.
[3]: The stated duration of toxicity is a minimum.

Table 4. Summary of chlorpyrifos use in counties where toxicity was observed.[1]

County	Month	Pounds of Chlorpyrifos Applied	3 Primary Uses
San Joaquin	January	118.4	Walnut Alfalfa Structural Pest Control
	February	687.3	
	March	8847.9	
	April	1061.8	
	May	9049	
	June	3600.6	
	July	11050.4	
	August	7755.3	
	September	2613.7	
	October	68.6	
	November	1	
	December	3.4	
Solano	January	1120.5	Walnut Alfalfa Structural Pest Control
	February	147	
	March	1994.5	
	April	1062.5	
	May	1163.7	
	June	170.6	
	July	4786.8	
	August	3355.4	
	September	456.5	
	October	9.1	
	November	61.7	
	December	1.5	
Stanislaus	January	3001.7	Almond Walnut Corn (forage-fodder)
	February	604.2	
	March	1454.7	
	April	721.8	
	May	12216.3	
	June	5300.3	
	July	13033	
	August	5455.4	
	September	922.2	
	October	129	
	November	0	
	December	641.2	

County	Month	Pounds of Chlorpyrifos Applied	3 Primary Uses
Yolo	January	114.2	Alfalfa Walnut Uncultivated Agriculture
	February	375.2	
	March	7135.3	
	April	227.9	
	May	950	
	June	319.9	
	July	5727.3	
	August	5613.9	
	September	2216.8	
	October	151.6	
	November	64.1	
	December	0.1	

[1]Data provided by Department of Pesticide Regulation, 2002.

3.1.1.5 Supporting Experiments

We were perplexed with the observations that air stripping reduced *C. dubia* mortality in samples that TIE procedures and chemical analyses clearly identified chlorpyrifos as the cause of toxicity. Therefore, we conducted a small experiment to determine if air stripping could affect chlorpyrifos toxicity to *C. dubia*. Laboratory control water was spiked with 2.5 TUs of chlorpyrifos. A sub-sample of this sample was air stripped. The aerated sub-sample, a non-aerated sub-sample and a methanol rinse of the aeration cylinder in control water, along with appropriate controls, were subjected to *C. dubia* testing. While the non-aerated sub-sample elicited statistically significant mortality, the aerated sub-sample did not (Volume II, Appendix B). Chlorpyrifos is not considered a particularly volatile chemical. Thus, we are suspect of inferences regarding the cause of toxicity based solely on the air stripping TIE procedure.

There have been speculations that agricultural drain waters contain substances (e.g., organic matter, particulates and other matter) that complex pesticides or other contaminants rendering them non-toxic. Thus, we were interested whether agricultural drain water might contain constituents that attenuate chlorpyrifos toxicity. Therefore, laboratory control water and a non-toxic agricultural drain sample (Sycamore Slough at Hwy 45 collected on 11 November 2003) were spiked with 20, 40, 60, 80, 100 and 120 ng/L chlorpyrifos. Results of this preliminary experiment revealed that chlorpyrifos was more toxic in drain water than in 'clean' laboratory control water (Volume II, Appendix B). In this report chlorpyrifos TUs were calculated based on the insecticide's LC_{50} in laboratory control water. The results of this experiment indicate that chlorpyrifos TUs could have been underestimated. We are uncertain as to the characteristics of agricultural drain water that potentiated chlorpyrifos toxicity relative to laboratory control water. Possibly there were other toxicants in the drain sample at sub-lethal concentrations that acted additively or synergistically with chlorpyrifos. Another possibility is that some drain water quality characteristics promote toxic effects of this insecticide. Follow-up on this simple experiment is most certainly needed as the implications are of considerable concern.

3.1.2 *Pimephales promelas*

Larval *P. promelas* toxicity test results from samples collected during the preplanned events are summarized in Appendix IV, Table 1. One hundred and eighty-eight samples were collected and tested. None of the samples caused statistically significant larval fish mortality.

3.2 Quality Assurance

Quality assurance measures were included to ascertain the reliability of the data collected during this project. Various components of the quality assurance program are summarized below.

3.2.1 Positive Control Studies

Positive controls consist of control water amended with a known concentration of toxicant. These samples are used to determine whether or not the test organisms are responding typically to a known concentration of chemical.

3.2.1.1 Reference Toxicant Tests

Reference toxicant tests were conducted monthly between March 2002 and October 2003 to ascertain whether test organism sensitivity was consistent over time. The chronic LC_{50} or EC_{50} for each test species is plotted for 20 consecutive months; along with two standard deviations from the cumulative and the running mean (Appendix V, Figures 1 through 8). In addition, the performance of the control organisms is plotted for each species. The US EPA (2002) identifies outliers as a data point falling outside of two standard deviations from the cumulative mean. The UCD ATL uses two standard deviations from the running mean to assess changes in animal sensitivity as they occur. Regardless of the type of standard deviation (cumulative or running) used to define the upper and lower limits, one data point can fall outside of these limits by chance alone. Two data points fell outside of the lower limit in the chart plotting control survival for *P. promelas* (Appendix V, Table 5). The survival for these two data points, 90 and 85%, is considered acceptable control performance for the chronic EPA toxicity tests and therefore, should not affect the reliability of this data. One or fewer data points fell outside of the two standard deviation limits for each species in the remaining reference toxicant control charts suggesting that test species response/sensitivity was within the acceptable range during this project.

In addition, acute reference toxicant tests were performed during the duration of the sampling period. The acute data are not expected to statistically identify outlying data points due to the limited sampling size. These graphical data can, however, illustrate trends in animal sensitivity (Appendix V, Figures 9 to 12).

3.2.1.2 Toxicant Spiked Laboratory Control Water

One toxicant-spiked laboratory control water was included as a quality assurance sample and was tested along with the samples collected during a preplanned sampling event. These tests are another means of assessing test species responsiveness/sensitivity. *C. dubia* and *P. promelas* were exposed to approximately one TU of diazinon and NaCl, respectively. Mortality in the spiked samples was statistically different compared to laboratory controls. Further, mortality was 100% within 96 hours suggesting that test organisms were responding typically to the toxicants.

3.2.2 Precision

Laboratory control duplicates, field duplicates and matrix spike duplicates were collected and tested with *P. promelas* and *C. dubia* to assess precision. Precision is the degree of agreement in measurements of the same characteristic between a sample and a duplicate sample. Twenty-two samples were processed to evaluate precision during this project. Precision for toxicity tests is calculated as the percentage of duplicates in agreement. Duplicate laboratory controls or trip blanks are in agreement when they do not differ statistically. Duplicate field samples or duplicate matrix spikes in agreement will either be both statistically different from the laboratory control or both be statistically similar to the control. Table 5 presents the number of duplicate samples in agreement for each species.

Table 5. Frequency of quality assurance duplicates sharing equivalent results.

Quality Assurance Samples	96-hour *C. dubia* tests		96-hour *P. promelas* tests	
	Sample Size	% in Agreement	Sample Size	% in Agreement
Laboratory Control Duplicates	6	100	6	100
Trip Blanks	8	100	7	100
Field Duplicates	8	100	7	100
Toxicant-Spiked Duplicates	1	100	1	100

Precision for water quality measurements is calculated as the relative percent difference.

$$\text{Relative Percent Difference} = 100 \times \frac{|\,\text{Duplicate \#1} - \text{Duplicate \#2}\,|}{(\text{Duplicate \#1} + \text{Duplicate \#2})/2}$$

The average relative percent difference is presented for each water quality parameter in Appendix V, Table 1.

3.2.3 Deviations and Corrective Actions

Six deviations and three corrective actions occurred during this project. Protocol deviations were issued when ATL staff did not follow Standard Operating Procedures. Corrective actions describe the measures taken to correct a deficiency or prevent the deviation from reoccurring.

3.2.3.1 Sample Receiving Temperatures

Samples are immediately cooled on ice following collection to preserve the integrity of the sample. After the ambient air and water temperature increased for the summer season, sample receiving temperatures were elevated for a period of 7.5 weeks. Sample receiving temperatures were cooler than the field temperatures; however temperatures did not reach the desired receiving temperature of less than 10ºC. In subsequent sample collections, more ice was placed in each cooler as a corrective action. The remaining samples collected for the project were received at temperatures below 10ºC.

3.2.3.2 Turbidity

Turbidity measurements only were taken for 71% of the samples. The samples collected between 22 May and 24 June 2003 do not have turbidity data due to technician oversight. After recognition of the missing data, turbidity measurements were initiated as a corrective action.

A linear regression between log transformed turbidity measurements and combined log transformed TSS and SSC datasets (Appendix V, Figure 13) showed a strong correlation between turbidity and quantifications of suspended solids (linear regression, $r^2 = 0.723$, $N = 132$, $P < 0.0001$). This result indicates that TSS/SSC is a good estimator of turbidity in the agriculture-dominated waters of the Central Valley. Since turbidity measurements are less costly and more time-efficient than either TSS or SSC procedures, we recommend turbidity be used as the primary measurement of suspended solids for samples from these waters, unless weight-of-evidence considerations demand a direct quantification of the mass of suspended solids present in a sample.

3.2.3.3 Re-sample

A deviation was issued in one instance where the test organisms were accidentally disposed of for a sample collected from Unnamed Drain @ Pomelo Rd. (Site 11) on 22 July 2003. Immediate re-sampling at this site served as a corrective action.

3.2.3.4 48 Hour Follow-up Sampling Time

On one occasion, a deviation was issued because ATL staff was unable to re-sample Westport Drain at Jennings Rd. (Site 10), originally collected on 22 July 2003, within the 48-hour limit. The re-sample was collected on 25 July 2003, within 72 hours.

3.2.3.5 Test Organisms

A deviation was issued on four occasions, 20 August 2003, 22 August 2003, 17 September 2003 and 24 September 2003, when the *C. dubia* used in the toxicity tests came from the mass cultures, rather than a brood board. In each instance, the control performance of the test was acceptable suggesting that these organisms were of adequate health for toxicity testing.

3.2.3.6 Ammonia

The 24-hour holding time for ammonium analyses was exceeded for one sampling event (25 September 2003) because the ATL ran out of a chemical reagent used in this analysis. Samples were analyzed 48 hours later when the reagent was received. Ammonia measurements are not expected to be significantly lower for this extended holding time.

3.3 Water Quality Parameters at Sampling Sites

Water quality parameters were measured at sampling sites during this project for four reasons: 1) to assist in characterizing water quality of agricultural drains, 2) to determine if individual water quality parameters were within the physiological tolerances of test organisms, 3) to identify if individual water quality measurements were within the numerical water quality criteria and 4) to aid in toxicity testing interpretation. The water quality data collected by UCD ATL staff at the sampling sites include temperature, dissolved oxygen, pH, ammonia, hardness, alkalinity, turbidity, total suspended solids (or suspended sediment concentration) and specific conductance (SC). Tables summarizing water quality parameters for individual sampling events are provided in Volume II , Appendix D.

Table 6 summarizes the water quality parameter ranges (UCD ATL measurements) for all samples collected during this study. Individual samples falling outside of the physiological tolerances of the test organisms are discussed within the parameter specific sections below. Dissolved oxygen and temperature are controlled during toxicity tests to ensure that these two parameters fall within the test organism's tolerance range. Specific conductivity, TSS and turbidity are of particular concern because of potential impact to aquatic species, including test organisms. That is, these parameters can confound interpretation of the toxicity testing results. These three parameters will be discussed in greater detail.

Dissolved Organic Concentrations (DOC) and Total Organic Concentrations (TOC) were measured in the UCD laboratory of Tom Young for all samples collected during this project (Appendix VI, Tables 19 and 20). Relatively speaking organic carbon at most of the sites was relatively high. Dissolved oxygen, conductivity, and pH were also determined in all these samples by the same laboratory (Appendix VIII). Organic compounds including alkylphenolethoxylates (known to be endocrine disruptors in fish), phthalates, and polycyclic aromatic hydrocarbons were detected in several samples analyzed in Tom Young's UCD laboratory.

Table 6. Summary of water quality ranges for preplanned sampling events, the special study and follow-up samples from 26 March 2003 to 7 October 2003.

Parameter	Range
Temperature (°C)	14.9 - 37.2
Dissolved Oxygen (mg/L)	1.35 - 15.5
pH	6.4 - 9.1
Ammonia (mg/L)	0 – 6.20
Hardness (mg/L as $CaCO_3$)	16 – 960
Alkalinity (mg/L as $CaCO_3$)	32 – 514
Turbidity (NTU)	2 - 298
Total Suspended Solids (mg/L)	0.26 – 1337
Conductivity (µS/cm)	60 – 3971

Various water quality criteria have been developed to protect specific surface water beneficial uses (CVRWQCB, 2003). National Ambient Water Quality Criteria were developed by US EPA (1986 and 2002b) under Section 304(a) of the Federal Clean Water Act to protect human health and aquatic life from pollutants in freshwater surface waters. These criteria provide guidance to states in development of water quality standards.

3.3.1 Temperature

Temperature was measured at each site and during all sampling events. The range temperatures for each site is provided in Appendix VI, Table I. Temperature for individual samples is presented in Appendix II, Table 2 to 3 and Appendix VI, Table 2. No specific water quality criteria have been established; however, surface water temperature must support successful fish migration, spawning, egg incubation and fry rearing of important species (SWRCB, 1971).

3.3.2 Dissolved Oxygen

The range of DO measurements at each site is summarized in Appendix VI, Table 3. The DO for individual samples is provided in Appendix II, Table 2 to 3 and Appendix VI, Table 4. DO measurements in samples collected at all sites determined in the Tom Young UCD laboratory are summarized in Appendix VIII. Seventy-seven percent of the sites had individual dissolved oxygen measurements below the US EPA National Recommended Water Quality Criteria (1-day minimum of 5.0 mg/L) to protect early life stage fishes in warm freshwater (US EPA, 1986 and 2002). The cause of low dissolved oxygen at these sites is unknown. Of note was low DO at sites with high organic carbon.

3.3.3 pH

The range of pH measurements at each site is provided in Appendix VI, Table 5. Individual pH measurements for each site and each sampling event are presented in Appendix II, Table 2 to 3 and Appendix VI, Table 6.

The Federal and California drinking water standards for pH are 6.5 to 8.5 (CVRWQCB, 2003). Agriculture water quality limits are 6.5 to 8.4 (CVRWQCB, 2003). The samples collected from each site had an average pH that fell within these criteria. However, pH in several individual samples fell outside of these criteria. Samples from Return Irrigation Drain at MCD Rd. (Site 3), Lone Tree Creek at Newcastle Rd. (Site 7), Wathal Slough at Woodward Ave. (Site 9) and Sand Creek at Miller Rd. (Site 21) fell below the lower pH limit for both criteria. Samples from Little John Creek at Newcastle Rd. (Site 8), Unnamed Drain at Pomelo Rd. (Site 11), Drain at Robben Rd. (Site 12a), Drain at Ulatis Creek at 113 (Site 13) and Sycamore Slough at 45 (Site 22) exceeded the upper limit for the Federal and California drinking water standards and the agricultural water quality limits. A single sample from Lone Tree Creek at Newcastle Rd. (Site 7) exceeded the agricultural limit, but not the drinking water standard.

3.3.4 Ammonia and Total Ammonia-Nitrogen

Un-ionized ammonia (NH_3) is more toxic to aquatic organisms than the ammonium ion (NH_4^+). Total Ammonium concentrations were converted to un-ionized ammonia concentrations using laboratory pH and temperature data from each sample. The range of ammonia concentrations for all samples collected at each site is summarized in Appendix VI, Table 7. The individual ammonia measurements for each sample appear in Appendix II, Table 2 to 3 and Appendix VI, Table 8. The *C. dubia* 48-hour LC_{50} for un-ionized ammonia is 1.82 mg/L at pH 9 and 1.42 mg/L at pH 8 (US EPA, 1993). All concentrations of ammonia were well below this concentration suggesting that ammonia did not cause or contribute to *C. dubia* mortality in any sample. The numeric water quality criteria for total ammonia-nitrogen (NH_3-N) are dependent on pH and temperature for the surface water site (US EPA, 2002). No samples exceeded the maximum 1-hour average for total ammonia-nitrogen established in the US EPA National Recommended Quality Criteria to protect aquatic life.

3.3.5 Hardness

Hardness was measured to determine the sum of calcium and magnesium in samples. Many agricultural drain samples were characterized by high hardness. The hardness range for each site is provided in Appendix VI, Table 9. Hardness for each individual sample can be found in Appendix

II, Table 2 to 3 and Appendix VI, Table 10. Water quality criteria have not been developed for hardness; however, hardness data can help identify water quality criteria exceedances for metals. Freshwater aquatic life criteria for some metals are expressed as a function of hardness because hardness affects the toxicity of metals. Increasing hardness concentrations reduce the toxicity of some metals.

3.3.6 Alkalinity

Alkalinity was measured to characterize the acid-neutralizing capacity in samples. The range of alkalinity concentrations for each site appears in Appendix VI, Table 11. Alkalinity for individual samples is available in Appendix II, Table 2 to 3 and Appendix VI, Table 12. Water quality criteria for alkalinity have not been developed.

3.3.7 Turbidity

Turbidity was measured to determine the clarity of samples. The range of turbidity at each site is summarized in Appendix VI, Table 13. Individual measurements for each site and sampling event are provided in Appendix II, Table 2 to 3 and Appendix VI, Table 14. The static-renewal toxicity tests conducted in this investigation are not designed to determine the effects of turbidity on aquatic biota. However, turbidity of toxic and nontoxic samples is illustrated in Figures 5 and 6. There was no association between turbidity and *C. dubia* mortality. Turbidity of toxic samples tended to occur at the lower or upper extreme of data points at each site. We cannot explain this pattern, but suspect that it relates to irrigation regimes.

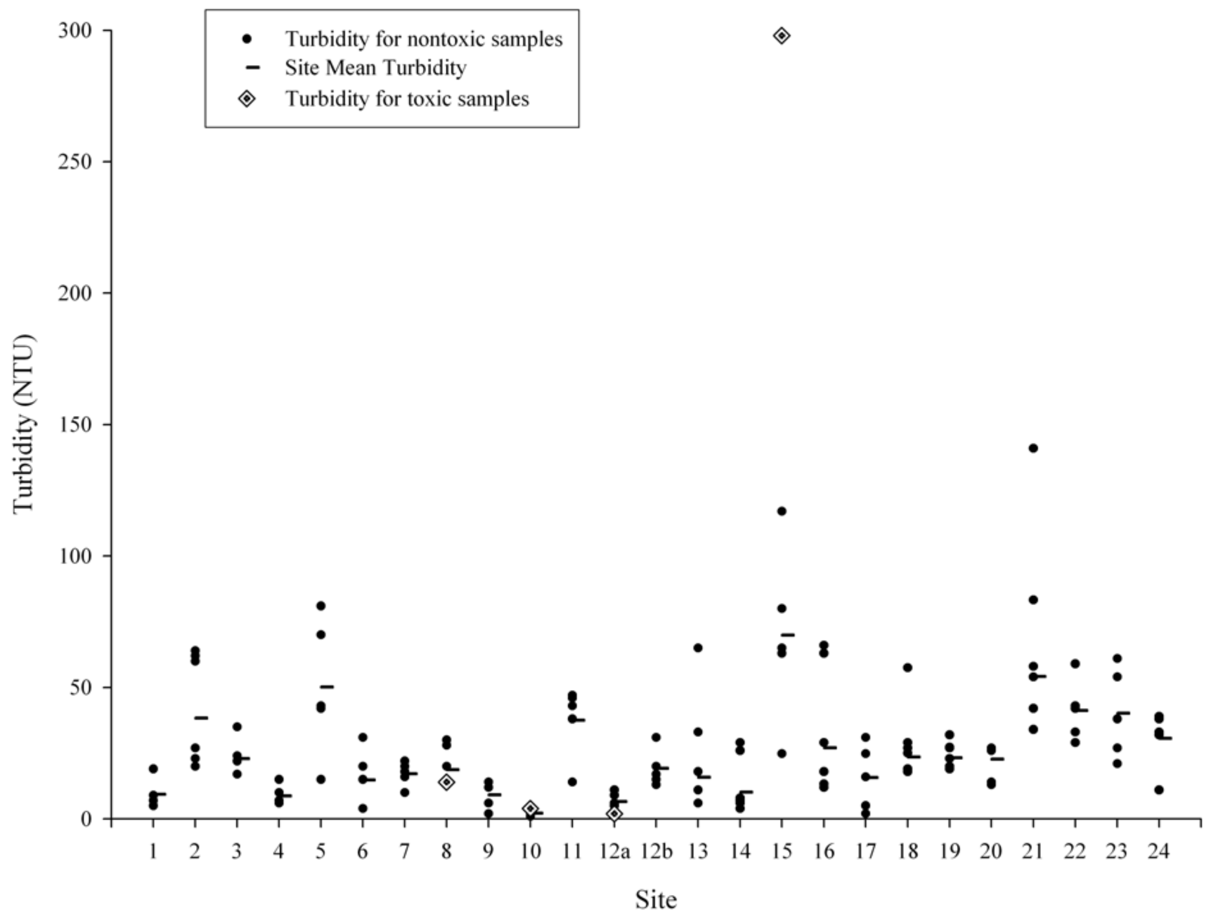

Fig. 5. Turbidity at sites during the irrigation return flow project. Each point represents turbidity on different collection dates. See Table 1 for site locations.

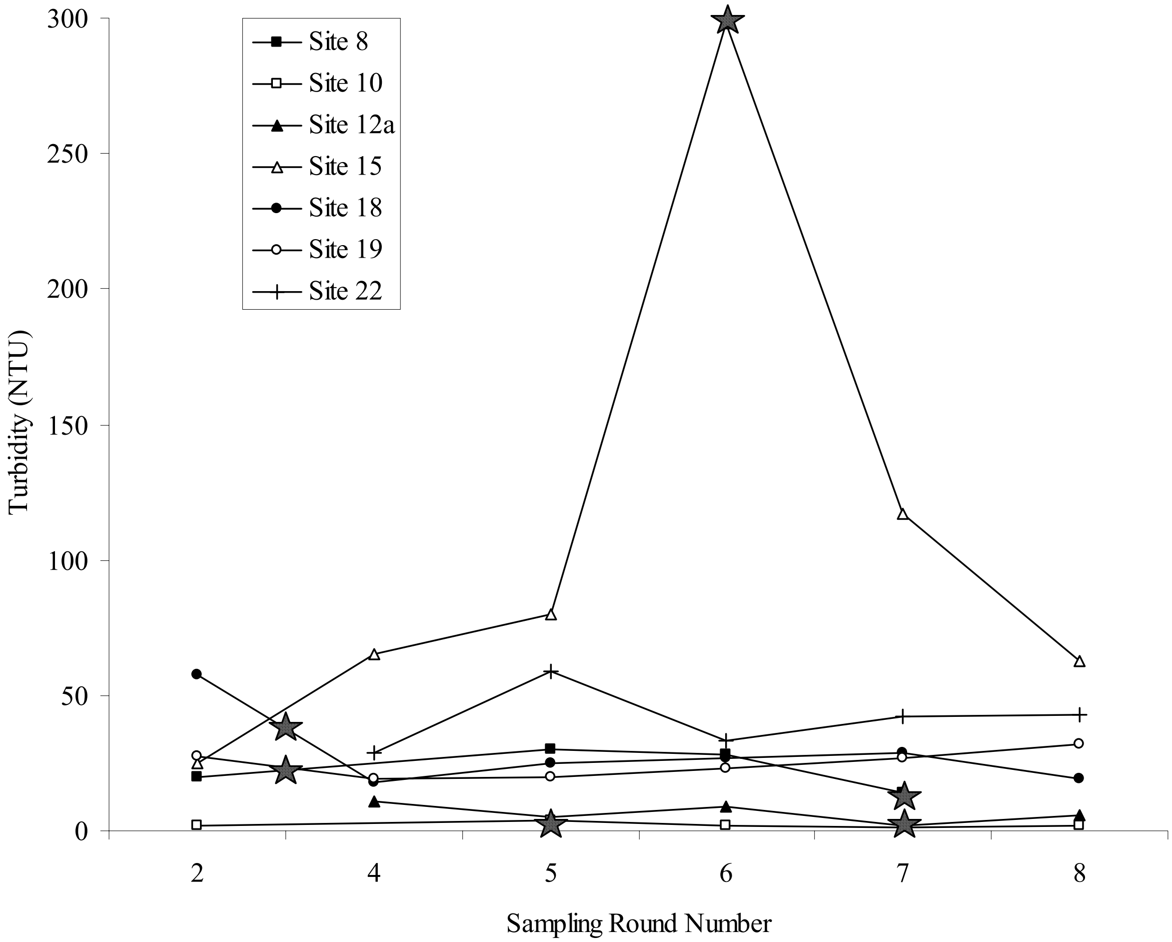

Fig. 6. Turbidity variation during the project at sites where toxic events were observed. Toxic samples are indicated by stars. See Table 2 for dates of sampling events.

The CVRWCB Basin Plan includes water quality criteria for turbidity. Exceedances of these criteria are on a sliding scale depending on the natural turbidity of the waterway. The average turbidity was calculated for each site over the duration of the project and is shown in Figure 5 and in Appendix VI, Table 13. The majority of sites had turbidity variations that exceeded Basin Plan requirements based on these averages. Additional studies should be conducted to evaluate turbidity criteria exceedances based on appropriate averaging periods (CVRWCB, 1998). The maximum turbidity for the Federal and State Drinking Water Standards is 115 NTU. No average turbidity for individual sites exceeded this criterion. Individual samples from two sites, Gordon Slough at Rd. 19 (Site 16) and Sycamore Slough at Hwy 45 (Site 22), exceeded this limit.

3.3.8 TSS/SSC

At the onset of this project, UCD ATL analyzed suspended solids using a Suspended Sediment Concentration (SSC) method. Due to high sediment concentrations in a majority of drain samples, filters clogged soon after initiating filtration, resulting in long delays to completion. As an alternative, a Total Suspended Solids (TSS) method was used. Table 7 summarizes the results of an experiment that compared the methods. Mean measurements were not significantly different between the methods (paired t-test, $t = -1.151$, $df = 4$, NS) and neither method was more precise than the other. Since TSS and SSC measurements were not significantly different and the units are the same, data collected with the two methods were combined.

Table 7. Comparison of TSS and SSC measurements.

		SSC			TSS		
Sample	Site Description	Mean	S.D.	% C.V.	Mean	S.D.	% C.V.
12a	Drain @ Robben Rd.	19.7	0.8	4.1	19.0	0.9	4.7
12a	Drain @ Robben Rd. F.D.	19.0	1.7	8.7	21.2	0.3	1.5
12b	Drain @ Robben & Midway Rds.	19.0	2.6	13.4	17.0	1.9	11.3
13	Drain @ Ulatis Creek & Hwy. 113	40.0	3.4	8.4	17.0	2.6	6.9
14	Creek @ Hawkins Rd.	15.0	2.4	15.7	13.6	1.1	8.2

The range of TSS/SSC for each site is summarized in Appendix VI, Table 15. TSS/SSC measurements for individual samples appear in Appendix II, Table 2 to 3 and Appendix VI, Table 16. TSS/SSC for toxic and non-toxic samples are shown in Figures 7 and 8. Range of TSS/SSC for toxic and nontoxic samples was 9 to 1196 mg/L and 0.26 to 1337, respectively. Thus, TSS/SSC for toxic samples fell well inside the range for nontoxic samples suggesting that suspended solids were not the cause or contributor of *C. dubia* mortality in any toxic sample. As with turbidity, TSS/SSC in the four toxic samples tended to appear at the low and high extreme of data points at each site. Suspended solids can affect sample toxicity by adsorbing hydrophobic compounds, rendering them less bioavailable to the test organism. Chlorpyrifos is a relatively hydrophobic compound. Nonetheless, high mortality was observed in samples collected from Westport Drain at Jennings Rd. (Site 10) and Lateral to Gordon Sl at Rd. 19 (Site 15) in the presence of high TSS/SSC concentrations. As indicated above, UCD ATL settles samples prior to testing, drawing test water from the top of the holding container. Furthermore, TIEs confirmed that chlorpyrifos was the cause of mortality in the site 10 samples. No water quality limits exist for suspended solids.

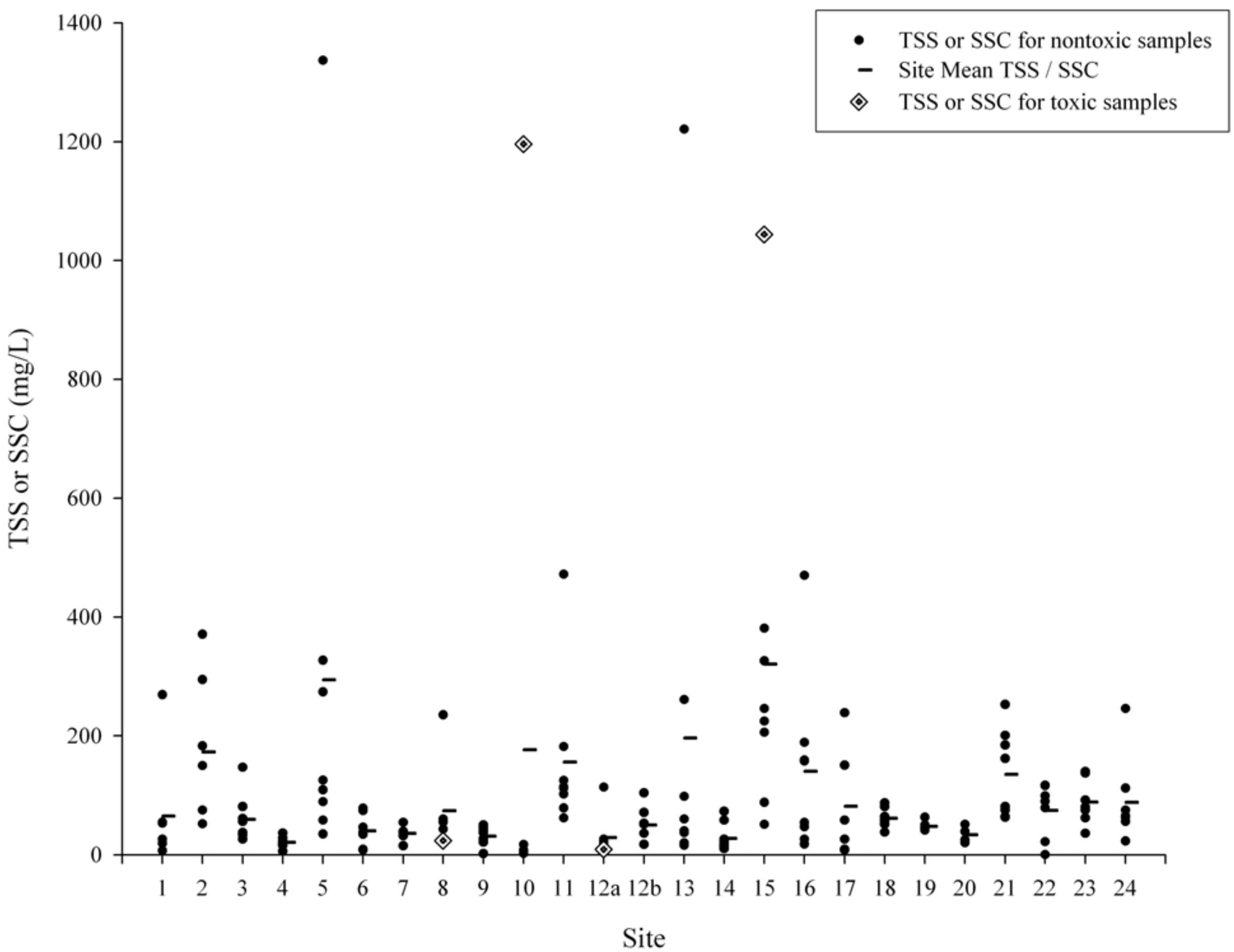

Fig. 7. TSS and SSC at sites during the irrigation return flow project. Each point represents suspended solids measurements on different collection dates. See Table 1 for site location.

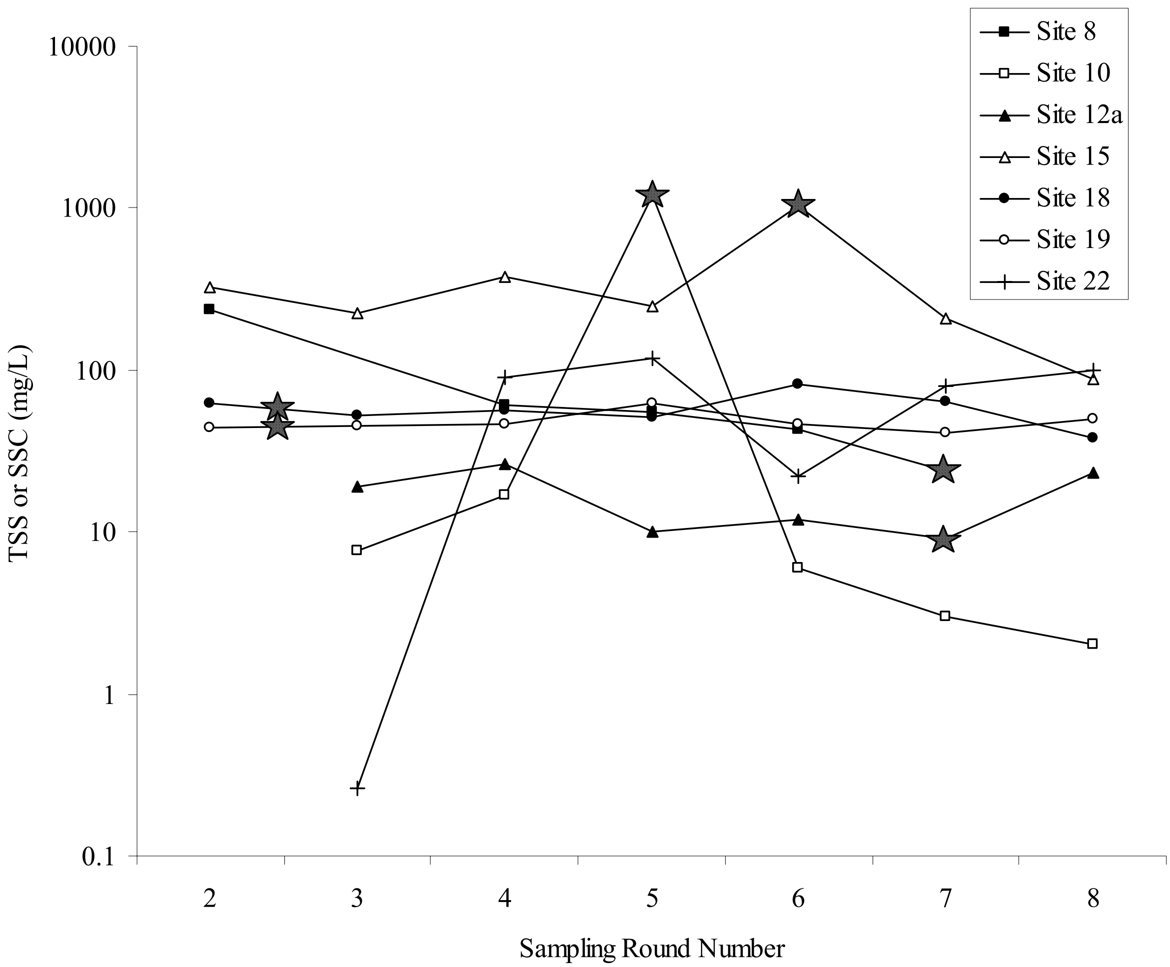

Fig 8. TSS/SSC variation during the project at sites where toxic events were observed. Toxic samples are indicated by stars. Note the logarithmic format of the TSS/SSC axis. See Table 2 for dates of sampling events.

3.3.9 Specific Conductivity

The SC range at each site can be seen in Appendix VI, Table 17. Conductivity measurements for individual samples appear in Appendix II, Table 2 to 3 and Appendix VI, Table 18. The UCD ATL has determined that the No Effect Concentration for NaCl is approximately 2500 µS/cm in 96-hour *C. dubia* exposures. Samples above 2500 µS/cm are diluted to bring the conductivity back within the organism's tolerance. Two samples from the drain at Bowman Rd. (Site 6) were characterized by conductivities higher than 2500. Sample dilution to reduce conductivity also reduces concentrations of chemicals that may have been toxic at their undiluted concentration. Therefore, an underestimation of toxicity is expected in diluted samples. Specific conductivity was measured at sites where the ten toxic samples were collected. Figures 9 and 10 illustrate SC of toxic and nontoxic samples. No association between SC and cladoceran mortality could be detected. The range of SC measurements for toxic samples (125 to 1032 µS/cm) fell within the range of nontoxic samples (59 to 3971 µS/cm) suggesting that conductivity was not the cause of or contributor to *C. dubia* mortality.

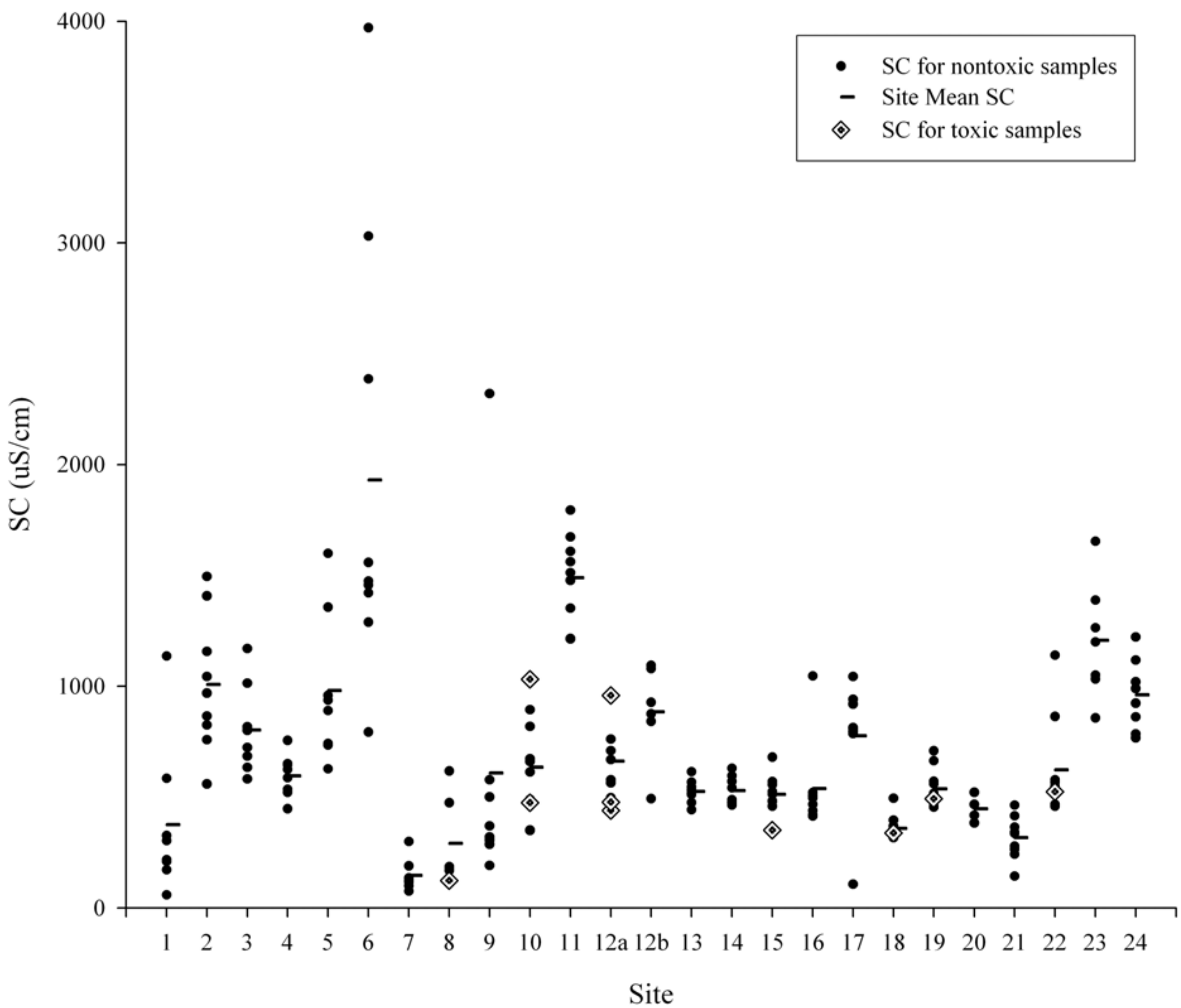

Fig. 9. SC at sites during the irrigation return flow project. Each point represents SC on different collection dates. See Table 1 for site location.

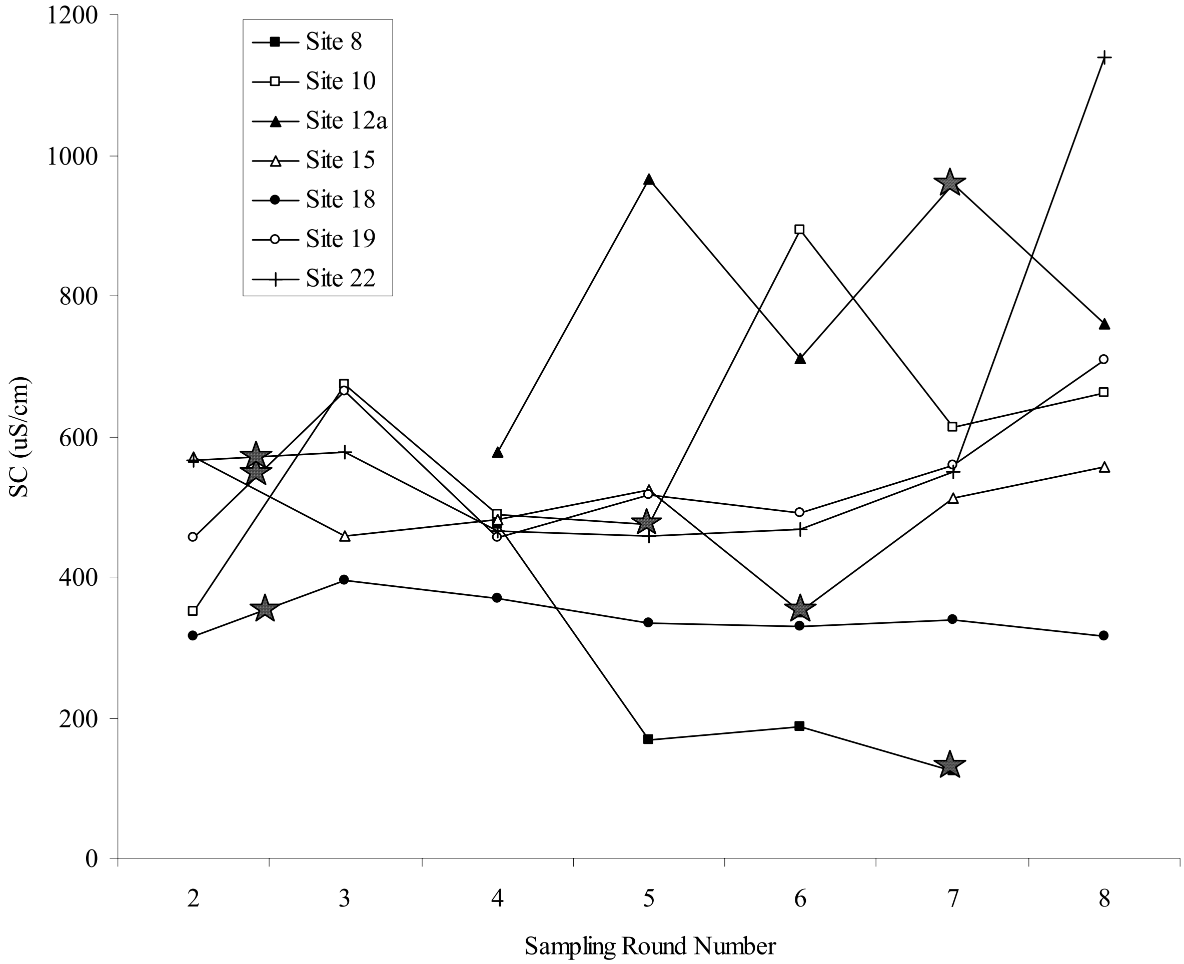

Fig 10. SC variation during the project at sites where toxic events were observed. Toxic samples are indicated by stars. See Table 2 for dates of sampling events.

As with turbidity and TSS/SSC, conductivity measurements of toxic samples tended to occur at the low or high end of data points at each site. As stated above, we suspect, but have no confirming data, that this pattern relates to irrigation regimes. Studies are needed to explore the potential relationship between irrigation patterns and aquatic species toxicity.

The Central Valley Regional Water Quality Control Board (CVRWQCB) has EC water quality criteria for the Sacramento River that range from 230 to 340 µS/cm. The average EC for the Sacramento River is 100 µS/cm. Drinking water criteria for EC are generally around 1500 µS/cm. EC water quality criteria for the protection of aquatic life need to be established. EC in a majority of agricultural drain samples was above 450 µS/cm (range=136 to 1795). Agricultural drain waters discharged into the Sacramento and San Joaquin Rivers may, therefore, be of concern to the CVRWQCB (1998).

3.3.10 Metals Data

Metal and ion concentrations were periodically measured (UCD laboratory of Tom Young) in samples collected at Sites 5, 11, 12, 18, 19, 22, and 23. These data are summarized in Appendix VIII.

Selenium and copper were the only metals that exceeded aquatic life water quality objectives in the Regional Board Water Quality Control Plan. Selenium exceeded the Basin Plan objective (5µg/L) at site AD23 on June 5 and 19, July 10, August 21, and September 11. Dissolved copper exceeded the hardness-based Basin Plan objective at site AD18 on June 11 and 16 and at site AD19 on June 11.

Sample concentrations of iron, manganese, barium, and aluminum exceeded Basin Plan drinking water objectives at the seven sites where analyses were performed on several occasions/sampling dates (Appendix VIII, Table 9).

3.3.11 Additional Field Data

Habitat data, velocity and flow were recorded at each site to provide additional information for risk assessment. These field data do not directly affect toxicity testing results because these parameters are controlled in laboratory testing. Habitat Assessment Field Data Sheets for Low Gradient Streams, developed from EPA were used to score habitats (Barbour *et al.*, 1999). Average habitat scores for each site are summarized in Appendix VII, Figure 1. Generally, poor or moderate habitat scores characterized most sites. The range of velocity data for each site is located in Appendix VII, Table 1. The velocity for individual sampling events is presented in Appendix II, Table 2 to 3 and Appendix VII, Table 2. Toxic samples tended to occur in conjunction with higher velocities at a site, except for the Drain at Robben Rd (Site 12a) where the pattern is the reverse. Again, we suspect this pattern relates to irrigation patterns. Flow was calculated from the velocity (m/s) at sites where cross sectional area (m^2) could be measured (Appendix II, Table 2 to 3 and Appendix VII, Table 3). Digital photographs were recorded at each site for all sampling events. These photographs were provided to CVRWQCB staff on cd and are presented in Volume II Appendix A.

4. Discussion

Results of this project further confirm that agricultural runoff of chlorpyrifos is a definite water quality degradation problem in California (e.g., de Vlaming *et al*., 2000; Werner *et al*., 2000; Anderson *et al*., 2002, 2003a, b; Hunt *et al*., 2003; de Vlaming *et al*., 2004a; Phillips *et al*., 2004). We propose that this problem be addressed in the interest of protecting and restoring water quality in agriculture-dominated waterways. An experiment conducted during the course of this project demonstrated that chlorpyrifos toxicity in a non-toxic agricultural drain matrix was more toxic (i.e., lower LC_{50}) than in laboratory control water. This observation requires follow-up consequent to implications of the observation.

Utilizing acute toxicity testing with two surrogate species as indicators of water quality, few instances of toxicant-degraded water quality in Central Valley agricultural drains were detected in this project. Based on these data *alone* one would predict that irrigation runoff and agricultural practices have relatively low impacts on water quality and biological condition in agricultural drains and agriculture-dominated waterways. Absence of acute toxicity in water samples should not be interpreted as absence of impairments due to agricultural activities on water quality or biological condition (beneficial uses). Consequent to limitations in the monitoring method used for the current project (see below) we contend further investigations are necessary to adequately characterize the relationship between agricultural runoff and biological condition/health of Central Valley waterways. Caution should be applied to interpretation and projection of data from this project. Specifically, the data collected in one season should not be used to predict water quality conditions in agricultural drains or agriculture-dominated waterways in other years.

Limitations of the primary tool, acute toxicity testing, include: (1) The only response measured was mortality, no sub-lethal responses were measured; (2) Only two indicator species were included in the testing. This limits the ability to detect to the entire spectrum of water quality stressors; (3) The procedures used are capable of responding to an incomplete number of water quality stressors, not habitat, or other physical stressors and sediment impacts; (4) Only one unusual (cool and rainy into May) irrigation season was included; (5) Sampling was not specifically event-based (associated with agricultural chemical use or peak irrigation regimes, etc.); (6) Sampling was too infrequent; (7) Geographic range of sampling sites was relatively limited; (8) Neither cumulative effects nor bioaccumulation effects were scrutinized, and (9) Most sampling sites were at the 'bottom' of large drains. These limitations restrict our ability to effectively assess or predict, based on the scant data collected, effects of irrigation runoff on water quality and on biological community integrity and health in agriculture drains and agriculture-dominated waterways. Nonetheless, an abundance of evidence (see summaries below) documents that several aspects of agricultural practices degrade water quality and impact biological communities. Clearly, a consistent, widespread and long-term monitoring and assessment program that applies a weight-of-evidence approach (see discussion below) is needed for agricultural drains and other agriculture-dominated waterways.

While toxicity testing has limitations (e.g., de Vlaming *et al*., 2000), results are not devoid of ecological meaning. In this investigation, laboratory toxicity tests on samples were used to evaluate water quality and to predict impacts on aquatic biota. The reliability of such extrapolations has been questioned (Hall and Giddings, 2000). However, several literature reviews (Waller *et al*., 1996; de Vlaming and Norberg-King, 1999; de Vlaming *et al*., 2001) conclude that toxicity test results are effective predictors of effects on ecosystem biota when appropriate considerations are given to exposure. Moreover, contaminant impacts on aquatic biota relates to the duration, magnitude and frequency of exposure. If the focus of a monitoring project is only on potential water quality effects on beneficial uses, we believe that toxicity testing, in association with TIEs, is the most informative monitoring approach, especially with limited budgets. Acute toxicity testing is a standard screening method and has been very effective in identifying worst-case water quality problems related to some contaminants. Further, when an objective is to investigate water quality at a large number of sites and/or have a high sampling frequency, in our opinion, acute toxicity testing can be the most informative and economical monitoring procedure. However, toxicity tests with sub-lethal endpoints, although more expensive, are more informative than acute tests.

Simple monitoring (single parameter), surrogate monitoring (use of surrogate/proxy data to infer aquatic ecosystem condition), and surveys can provide information on what is changing in the environment, but alone, are unable to answer the important question of why changes are occurring (Brydges, 2004). A much more detailed set of physical and ecological information is usually required to establish cause-and-effect. The concept of integrated monitoring has been developed with the overall objectives of recording changes in the environment and understanding and defining the reasons for these changes. Integrated monitoring is characterized by long-term multidisciplinary efforts that include physical, chemical, toxicological, and biological/ecological data (Brydges, 2004).

We recommend that future agricultural drain and agriculture-dominated waterway monitoring projects include more frequent sampling and physical, chemical, toxicological and biological monitoring procedures (i.e., multiple procedure/integrated monitoring approach), as well as a design that will enable assessment of geographic extent of possible effects. We recommend that toxicological procedures include water column testing with a wider range of test species and sub-lethal end points, sediment toxicity testing and *in situ* toxicity testing with resident species.

When designing a monitoring project it is essential to understand the capabilities and limitations of the primary procedures available. Summarized below are some capabilities and limitations of biological, toxicological, and chemical procedures.

1. Chemical-specific monitoring (e.g., US EPA, 1991)

Capabilities include:

- Analytical procedures can accurately quantify specific chemicals known to have adverse impacts on aquatic life beneficial uses.
- Analytical procedures for many chemicals are highly standardized with specific QA/QC requirements (high degree of accuracy and precision).
- Analytical procedures for many chemicals provide reliable, repeatable, and comparable results relative to bioassessments.

- Analytical procedures can furnish an early warning signal so that actions can be initiated to minimize impacts on beneficial uses.
- As a component of TIE procedures, analytical procedures provide a primary contribution to identification of the cause(s) of toxicity to aquatic life.

Limitations include:

- Chemical analyses do not assess chemical bioavailability.
- Interactions (e.g., additivity, synergism, antagonism) among contaminants are not accounted for.
- While ambient samples may contain a large number of contaminants, analytical procedures are usually not geared to measure all of them. Expanding analytical procedures to incorporate all potential contaminants would be costly.
- Water quality standards/criteria exist for only a small number of contaminants that potentially enter waterways.
- Analytical procedures do not characterize the persistence/duration or frequency of aquatic biota exposures without repeated sampling and analysis.

2. Toxicity testing (e.g., US EPA, 1991; de Vlaming and Norberg-King, 1999; de Vlaming *et al*., 2000; de Vlaming *et al*., 2001).

Capabilities include:

- Have the potential to provide integrative measure of aggregate toxicity of constituents in a sample.
- Toxicity caused by compounds commonly not analyzed for in chemical tests are identified by these tests.
- Provide a direct measure of contaminant bioavailability to aquatic species.
- In combination with TIEs, toxicity tests can identify the chemical cause(s) of toxicity.
- Toxicity tests are highly standardized with specific QA/QC requirements.
- Tests afford reliable, repeatable, and comparable results compared to biological assessments.
- Tests can furnish an early warning signal so that actions can be initiated to minimize ecosystem impacts.

- Tests are reliable qualitative predictors of biological community impacts when appropriate consideration is given to exposure.

Limitations include:

- Toxicity tests do not characterize the persistence/duration or frequency of aquatic biota exposures without repeated sampling and testing.
- Tests do not directly measure biotic community responses.
- Tests do not encompass the range of species sensitivities or functions responsive to toxic chemicals that occur in most biological communities.
- Neither delayed impacts nor effects due to bioaccumulation and bioconcentration are detected.
- The highly controlled exposure regimes in laboratory tests do not always reflect the multivariate and complex conditions in aquatic ecosystems. Toxicity testing results likely underestimate impacts to biotic communities because of multiple stressors acting on aquatic ecosystems.
- Tests fail to account for indirect effects of contaminants.

3. Bioassessment (e.g., Barbour *et al.*, 1996; Clements and Kiffnys, 1996; de Vlaming and Norberg-King, 1999; de Vlaming *et al.*, 2000; LaPoint and Waller, 2000).

Capabilities include:

- Bioassessments provide a *direct* measure of ecological condition of a site/waterway.
- Biological communities integrate the cumulative effects of multiple stressors (physical and chemical) over time and thus provide a holistic measure of aggregated impact.
- Biological community condition reflects both short- and long-term effects of stressors.
- Bioassessments can provide the only unequivocal/direct documentation that aquatic life beneficial uses are impacted/impaired.
- Bioassessment results tend to be more convincing and understandable to the public and legislators than chemical and toxicological monitoring data.

Limitations include:

- Many bioassessment studies fail to account for *natural* temporal (season to season and year to year) and spatial variations. This results in considerable difficulty distinguishing anthropogenic effects on biological community health. It is critical to differentiate natural variation (which can be considerable) from anthropogenic impacts on biological community health. Several years of data are necessary to effectively characterize natural temporal variation.
- Many bioassessment studies do not provide conclusions regarding impacts on impairment because reference (or least impacted) conditions are not determined. Biosurvey data cannot fully characterize aquatic life beneficial uses until reference conditions and biocriteria are developed.
- Many bioassessment studies do not include replication at a site so accuracy and precision of measurements is unknown.
- Most bioassessments are characterized by a high degree of variability because biological systems tend to be variable. High variability results in reduced ability to observe statistically significant differences between sites and in low procedure resolution/sensitivity (ability to discriminate test sites from reference sites/conditions).

While all three of these monitoring procedures have strengths and can be effective, appropriate design (e.g., site selection, type, timing and frequency of sampling, concurrent assessment for potentially significant physical, chemical, toxicological and biological parameters, and analyses applied to the collected data) as related to the objective(s) is critical.

This and other investigations document that water quality in agriculture-dominated waterways is temporally variable due to agricultural practices. That is, pulses of degraded water move through these waterways. Irrigation patterns and regimes are almost certainly involved in this phenomenon, in association with other variable agricultural practices. A more complete understanding of these irrigation practices in relation to water quality is essential. Further, the relationship of pulses of degraded water quality on biological condition and aquatic ecosystem health requires further investigation. Duration of a pulse of chlorpyrifos-caused toxicity was at least seven days at one site in Solano County. Such duration almost certainly would have impacts on biological communities. This drain, however, was relatively small. Pulses of degraded water quality vary with size and flow (waterway order) of waterway, smaller ones more likely to be characterized by transient water quality-degraded pulses. In this project, relatively infrequent sampling and only one sampling site per waterway precluded defining the duration and geographic extent of water quality degradation events. Such information is important to data interpretation and predictions.

4.1 Agricultural Land Use Related to Aquatic Ecosystems

The Central Valley Regional Water Quality Control Board funded an exploratory project with UCD ATL that applied benthic macroinvertebrate (BMI) bioassessment to agriculture-dominated waterways (ADWs) and effluent-dominated waterways (EDWs) (de Vlaming *et al*., 2004b). BMIs constitute an important link in freshwater aquatic ecosystem structure and function. The primary goal of that study was to assess BMI community structure and physical habitat conditions in several ADWs and effluent-dominated waterways of the Central Valley. An important aspect of the investigations was to identify environmental factors affecting BMI community integrity. Analyses identified agricultural land use as a likely determinant (negative relationship) of BMI community integrity (de Vlaming *et al*., 2004b). Impacted BMI communities and impaired aquatic and riparian habitat conditions characterized ADWs. Habitat (instream and riparian vegetation) conditions in ADWs were poor to marginal. Environmental variables identified as probable determinants of BMI community integrity included substrate, several physical habitat factors and some water quality variables. Downstream sites on ADWs tended to manifest more robust BMI communities than upstream sites surrounded by intense agricultural activities. That is, the most impacted sites were located adjacent to the highest intensities of agricultural activities. Of the environmental parameters measured, water quality parameters appeared to exert less effect on BMI community integrity than physical habitat factors. De Vlaming *et al*. (2004b) hypothesized that effects of water quality parameters were difficult to detect with the bioassessment procedure because physical habitat was so poor at most ADW sites. Moreover, it is not that water quality is acceptable, but rather that physical conditions are so poor that water quality degradation is difficult to detect with bioassessment procedures. ADWs manifested a range of biological conditions and de Vlaming *et al*. suggested that these waterways could support more robust BMI communities if physical habitat and water quality were not degraded.

Other investigators have examined the association of agricultural and urban land use with BMI community structure and metrics. Brown and May (2000) discovered that agricultural and urban land uses were strongly associated (negative correlation) with macroinvertebrate community structure and metrics in the lower San Joaquin River watershed. Relationships between land use/anthropogenic activities and water quality in the San Joaquin River plus its tributaries were assessed by Pereira *et al*. (1996). These investigators reported that suspended and bed sediments serve as sinks for hydrophobic pesticides. The hydrophobic insecticides are bioavailable and accumulate in lipid tissues of aquatic biota. Because of this bioaccumulation of insecticides and other agricultural chemicals, effects on aquatic and terrestrial food chains are probable. The effects of such bioaccumulation and biomagnification on aquatic and terrestrial biota are unknown, but could be considerable. The suspended insecticide-contaminated sediments were transported throughout the San Joaquin system, so there are potentially widespread impacts. Bioaccumulation is a definite issue in agricultural drains and ADWs, but was not investigated in the current project. Agricultural contributions to aquatic ecosystems of pesticides and other agricultural chemicals that bioaccumulate and biomagnify, as well as the probable effects of such phenomena, have been to a large extent ignored.

Griffith *et al*. (2003) examined relationships between environmental gradients and macroinvertebrate assemblages in the Central Valley portions of the Sacramento and San Joaquin River watersheds. According to these authors, the probable primary environmental determinants of BMI assemblages in the Central Valley are instream habitat, including substrate type: (1) By metrics analysis—channel morphology and substrate and (2) By taxa abundance analyses—specific conductivity, channel morphology and substrate. Channel management activities and landscape scale alterations of catchments by agriculture were identified by these authors as the major activities responsible for the environmental factors determining BMI assemblages.

Invertebrate community composition in two upstream reaches of a creek in Ontario, Canada was scrutinized (Dance and Hynes, 1980). Agricultural land use adjacent to the two creek reaches varied considerably. These investigators concluded that intensive agricultural land use had profound effects on benthic macroinvertebrate community integrity. Community integrity was more impacted in the stream reach with more intense agricultural practices than in the reach with less intense practices. Further, benthic macroinvertebrate communities were less diverse in agriculture-influenced streams than in an unmodified stream of similar size and substrate. These authors suggested the agriculture-related activities that most likely impacted biological communities were flow regime, stream channelization, sediment, temperature and water quality.

Three streams in the Piedmont ecoregion of North Carolina were studied to evaluate the effect of land use on water quality and aquatic biota (Lenat and Crawford, 1994). Land use around one stream was forest-dominated, another was urban-dominated and the third was agriculture-dominated. Only one site on each stream was sampled, but sites were sampled in January, April, June and November. The three streams differed in regards to BMI community structure. The stream surrounded primarily by forests was characterized by high BMI richness, especially intolerant EPT groups (dominant taxa—mayflies), many unique species and many intolerant species. Similar to our findings, the agriculture-dominated stream was characterized by low EPT taxa richness, many tolerant taxa and dominant populations of chironomid midges. Because land use in this watershed was 48% row crops and 31% forested, it is questionable that this stream effectively represented a purely agriculture-dominated watershed.

While the application of bioassessments to assess the effects of agricultural land use on aquatic ecosystem biological communities has been rather limited in California, bioassessments performed elsewhere document that farming activities degrade stream/river water quality and habitat, significantly impacting BMI communities (Kendrick, 1976; Welch *et al*., 1977; Schofield *et al*., 1990; Delong and Brusven, 1998; Sallenave and Day, 1991; Kay *et al*., 2001). Other biological assessment studies that provide evidence supporting this relationship are summarized in Section 4.4, below.

4.2 Loss of Aquatic Life Biodiversity

The central message in Karr and Chu's (1999) recent book titled 'Restoring Life in Running Waters' is the accelerated and pervasive degradation of aquatic biota and loss of biodiversity in the United States. A combination of stressors contributes to these losses including habitat loss and degradation, dams and water diversions, sediment and chemical contaminants, hydrological modifications, introduced non-native species and over exploitation. Precipitous losses of biodiversity and population declines in aquatic ecosystems are well documented (e.g., Christian, 1995). The greatest losses of biodiversity in the U.S. have occurred in California and Hawaii. Ricciardi and Rasmussen (1999) presented evidence that (1) freshwater biota in the U.S. are disappearing five times faster than terrestrial species and three times faster than coastal marine mammals, (2) extinction rates of freshwater animals are accelerating, and (3) North American freshwater biodiversity is being depleted at the same rate as that of tropical rain forests. Richter *et al*. (1997) concluded that the three leading threats to aquatic species are, in order (1) agricultural non-point pollution, (2) alien species, and (3) altered hydrologic regimes. Wilcove *et al*. (2000) proposed that the three leading causes of the decline of aquatic biota are, in order (1) habitat degradation/loss, (2) pollution, especially from agricultural origin, and (3) alien species. Wilcove and colleagues further concluded that 'the most overt and widespread forms' of aquatic ecosystem habit alteration are by agriculture. In a literature review, Cooper (1993) indicated that agriculture is a, if not the primary, major source of water quality degradation in the U.S.

4.3 Multiple Agriculture Stressors to Agricultural Drains and ADWs

Because of inherent limitations, care must be exercised when extrapolating laboratory results to predictions regarding impacts on natural aquatic ecosystems. Multiple stressors affect biota in waterways of the Central Valley (de Vlaming *et al*., 2004b) and elsewhere (e.g., Cooper, 1993). Extrapolations of laboratory data frequently underestimate impacts on aquatic biota because of multiple stressors and additivity of stressors. Aquatic species in Central Valley agricultural drains and ADWs are exposed to a mixture of chemicals and other multiple non-chemical stressors. Thus, traditional single chemical risk assessments are *not* realistic or very useful.

Single chemical probablilistic environmental risk assessments (PERA) have been applied to predict potential impacts of OP insecticides on aquatic biological communities, especially in California (Novartis Crop Protection, 1997; Giesy *et al*., 1999). If PERA data are not extended beyond assumptions used in their applications or beyond the limitations of the procedure, the information provided by this approach can be useful. However, while proponents for PERA promise a more realistic evaluation of potential risks to aquatic communities, serious limitations of PERA have been noted (de Vlaming, 2000; Kent, 2004). One primary limitation to PERA is that they are applied to single chemicals when aquatic biota are exposed to multiple other contaminants and other types of stressors.

Many agricultural activities and materials, including habitat destruction and modification (e.g., channel modification; instream habitat and riparian vegetation), hydrology modifications (e.g., variable flow regimes), sediment, pesticides and other agricultural chemicals, organic carbon and wastes, nutrients, salinity and turbidity have the potential to impact aquatic biological communities. Because of multiple stressors, water quality standards (criteria) alone are insufficient to protect or restore aquatic biological communities. Furthermore, agricultural drain and agriculture-dominated waterway monitoring programs and projects should encompass all stressors that potentially impact biological communities. A weight-of-evidence approach that integrates multiple procedures to assess physical, toxicological (including water column, sediment and *in situ*), chemical and biological condition of waterways would be most informative. Burton *et al*. (2002) published a review of the advantages, limitations and uncertainties associated with various weight-of-evidence approaches. The National Research Council (2001) strongly endorsed the weight-of-evidence approach to monitoring and assessment.

In situ toxicity tests with indigenous species have been particularly effective in demonstrating insecticide runoff effects on aquatic systems. Crane *et al*. (1995) applied a battery of *in situ* toxicity tests to assess the effects of runoff from agricultural lands on water quality in a steam in the United Kingdom. The major goal was to appraise whether the *in situ* tests would provide information that complimented BMI bioassessment and chemical monitoring data. Results of the *in situ* tests with an amphipod and a midge (larval dipteran insect) from the bioassessments were complimentary. Both revealed biological impacts of agricultural runoff. Transient runoff of carbofuran from an oilseed crop into a headwater stream draining treated farmland in the United Kingdom was shown, with *in situ* testing, to impact a gammarid amphipod crustacean (Matthiessen *et al*., 1995). *In situ* tests were employed by Tucker and Burton (1999) to investigate water quality in agriculture- and urban-dominated streams in Ohio. Of particular note, toxicity (related to runoff) to the invertebrate test species in the *in situ* tests was greater in the agriculture-dominated stream when compared to ambient water toxicity tests conducted in the laboratory. Results of *in situ* testing were in general agreement with BMI bioassessment data. Several other investigations (Schulz and Peall, 2001; Schulz *et al*., 2001; Moore *et al*., 2002; Schulz, 2003) have linked insecticides to water quality degradation and biological effects using *in situ* testing. Validity and ecological relevance of *in situ* tests has been addressed by Schulz and Liess (1999). Limitations of *in situ* testing include: (1) Typically only mortality is evaluated; (2) Results are less meaningful when highly mobile organisms are used; and (3) Care must be taken if results are used to predict effects on organisms that move with a mass of water (e.g., zooplankton species, larval fish, etc.).

4.4 Agriculture-related Water Quality Stressors

While all potential stressors on biological communities are of concern, the procedure used in this study has a water quality focus. Further, it is not the intent of this report to provide a literature review of all potential agriculture-related stressors on biological communities. For the most part, the following discussion focuses on potential chemical stressors.

In the current investigation chlorpyrifos was the primary chemical identified as causing water quality degradation. Chlorpyrifos is the most used insecticide in U.S. agriculture. In agricultural drains and other agriculture-dominated waterways, pesticides (especially insecticides) have the potential to degrade water quality and impact aquatic biota. Insecticide pollution is widely regarded as one of the greatest causes of contamination of surface waters (e.g., Line *et al*., 1997; Loague *et al*., 1998; Gangbazo *et al*., 1999). Benthic biological communities constitute a critical component of aquatic ecosystems. Insecticide contamination of sediment has the potential to impact benthic communities and, consequently, impact aquatic ecosystem health. Nonetheless, toxicity testing with sediments from agricultural drains and ADWs has been woefully neglected. Recently, Weston and colleagues (2004) published results of toxicity testing of sediments collected at sites located in agricultural drains and ADWs of California's Central Valley. Sediment samples from 42 percent of the sites caused significant mortality to test species. Pyrethroid insecticides appeared to be the primary cause of mortality at most of these sites. These findings are of considerable importance and underscore the need to expand sediment toxicity testing in Central Valley agricultural drains and ADWs.

Water quality degradation linked to chlorpyrifos and diazinon toxicity to aquatic invertebrates has been documented in several urban- and agriculture-dominated California watersheds (Bailey *et al*., 1996; Bailey *et al*., 2000; de Vlaming *et al*., 2000; Werner *et al*., 2000; Anderson *et al*., 2002, 2003a, b; Hunt *et al*., 2003; Phillips *et al*., 2004; de Vlaming *et al*., 2004a). Crustaceans and larval aquatic insects are particularly sensitive to chlorpyrifos (Giesy *et al*., 1999). Mortality was the primary response assessed in the current investigation as well as in the cited studies. Sub-lethal effects of lower concentrations of these OP insecticides have not received adequate attention. OP insecticides frequently co-occur in surface waters and their toxicity is additive (Bailey *et al*., 1997). The California Department of Fish and Game demonstrated concern regarding chlorpyrifos degradation of water quality by publishing water quality criteria for the protection of aquatic life (Siepmann and Finlayson, 2000). The acute and chronic exposure water quality criteria for chlorpyrifos are 20 and 14 ng/L, respectively.

A multiple procedure approach was applied to assess the effects of agricultural pollutants entering the Salinas River from a tributary draining an agricultural watershed (Anderson *et al*., 2003a, b; Hunt *et al*., 2003; Phillips *et al*., 2004). Data were collected at stations upstream and downstream of the agricultural input. Analyses included water column chemical analyses, water column toxicity testing with *Ceriodaphnia dubia* plus TIEs, sediment toxicity testing with *Hyalella azteca* (a resident species), *in situ* toxicity tests and benthic macroinvertebrate bioassessments. Downstream concentrations of chlorpyrifos exceeded the lethality threshold of *C. dubia* while upstream chlorpyrifos concentrations were low. Toxicity tests with *C. dubia* confirmed acute toxicity at downstream stations. The upstream station was non-toxic. Sediment samples downstream of the creek also were toxic to *H. azteca* whereas sediment upstream of the agricultural input was not toxic.

Chlorpyrifos concentrations in the sediment collected downstream of the input exceeded the lethality threshold of this species. TIEs identified chlorpyrifos as the cause of toxicity. Benthic macroinvertebrate data revealed that downstream stations were impacted relative to upstream stations. All lines of evidence linked chlorpyrifos in the irrigation runoff dominated stream to impacts on Salinas River biota. Laboratory toxicity tests with *C. dubia* and *H. azteca* were predictive of benthic macroinvertebrate data and *in situ* test results.

The Alamo and New Rivers, located in the Imperial Valley, California receive large volumes of irrigation runoff and discharge into the ecologically sensitive Salton Sea. Between 1993 and 2002, UCD ATL conducted a series of studies to assess water quality in these systems using three aquatic species: a cladoceran (Ce*riodaphnia dubia*), a mysid (*Neomysis mercedis*) and a larval fish (*Pimephales promelas*). Although no mortality was observed with *P. promelas*, high-level toxicity to the invertebrate species was documented in samples from both rivers during many months of each year. Toxicity identification evaluations (TIEs) and chemical analyses identified the organophosphorus (OP) insecticides, chlorpyrifos and diazinon, as the cause of *C. dubia* toxicity. The extent of the *C. dubia* mortality was highly correlated with quantities of these OPs applied in the river watersheds. *C. dubia* mortality occurred during more months of our 2001/02 study than in the 1990s investigations. During 2001/02, the extensive *C. dubia* mortality observed in New River samples was caused by OP insecticide pollution that originated in Mexico. Mortality to *N. mercedis* in New River samples was likely caused by contaminants other than OP insecticides. UCD ATL studies documented pollution of the Alamo River caused by OP insecticides (chlorpyrifos and diazinon) over a 10-year period and provided information needed for remediation efforts.

In an investigation of the San Joaquin River watershed Leland and Fend (1998) proposed that invertebrate community structures were unrelated to 'pesticide distributions'. Further, the authors suggested that BMI communities are not likely susceptible to seasonal changes in concentrations of anthropogenic constituents. However, only some pesticide constituents were measured (on only two occasions during the three-year study). Sediment pesticide concentrations were not analyzed. Thus, we contend that their data on pesticides were much too incomplete to warrant the conclusions advanced by these authors. One objective of a study conducted by Hall and Killen (2001) on

Orestimba and Arcade Creeks was to assess potential impacts of OP insecticides, particularly chlorpyrifos, on BMI communities in these two streams. Hall and Killen concluded that habitat factors likely explained the differences in BMI communities and suggested that contaminants played a minor role. However, the Hall and Killen study did not include reference streams nor did they report instream insecticide concentrations or sampling site relationship to insecticide use. Associations of water quality factors including contaminants with BMI metrics were not evaluated, so contaminant effects, including chlorpyrifos, cannot be ruled out.

OP insecticide degradation of water quality is not restricted to California. The US Geological Survey's National Water Quality Assessment Program (NAWQA) has been monitoring major watersheds distributed throughout the US since 1991. NAWQA data reveal that concentrations of four OP insecticides (chlorpyrifos, diazinon, azinphos-methyl and malathion) exceed water quality criteria for aquatic life protection more than any other pesticides (Larson *et al*., 1997; Gilliom *et al*., 1999). These OPs originate from both agricultural and urban sources. McLeay and Hall (1999) reported that during the growing season the Nicomekl River (in British Columbia, Canada) appears to be periodically contaminated with OP insecticides.

Chlorpyrifos and diazinon degradation of water quality is being scrutinized by US EPA. As of July 2003, 62 waterways were designated as impaired by diazinon on the Clean Water Act §303(d) list (http://oaspub.epa.gov/pls/tmdl/waters_list.impairments?p_impid=3). All but three of these waterways are in California. Twenty waterways are impaired by chlorpyrifos. Of these waterways, 15 are in California, two in Washington and two in Maryland. That the majority of waterways identified as impaired by chlorpyrifos and diazinon are in California probably relates to the State having a more extensive surface water toxicity program than other States (de Vlaming *et al*., 2000). The California ambient water program includes application of TIE procedures to toxic samples. Thus, cause(s) of toxicity to aquatic species can usually be identified. Chlorpyrifos and diazinon are widely used in the US (pp. 160-161 and 190-194 in Larson *et al*., 1997). US EPA (1999) estimated that non-agricultural use of OP insecticides is over 17 million pounds per year and agricultural use accounts for another 60 million pounds. The impacts of OP insecticides on 150 endpoints in 20 aquatic species have been linked to tissue residues (Jarvinen and Ankley, 1999).

There is evidence that insecticides, including OPs, in agricultural runoff have significant impacts on benthic macroinvertebrate (BMI) communities. BMI community integrity is a critical component of healthy aquatic ecosystems. Liess and Schulz (1999) also documented that insecticides (ethyl-parathion and esfenvalerate) in runoff from agricultural lands had significant negative impacts on stream BMI communities. The effects of insecticides in runoff were distinguished from/independent of hydraulic stress, suspended particulates and nutrients. Recovery of BMI communities required six to 11 months. A noteworthy finding in this study was that BMI community assessments revealed more severe impacts than predicted by laboratory toxicity test results. A significant aspect of these two studies was that sampling was event-based. Determination of an association of insecticides in agricultural runoff with effects on BMI has perhaps been a methodological issue. Event-based sampling (e.g., sampling associated with peak irrigation after insecticide application) is more likely to define the effects of insecticides on BMI communities than is random or probabilistic sampling.

Increased BMI drift rate in streams following insecticide contamination has been confirmed in several studies (Cuffney *et al*., 1984; Scherer and McNicol, 1986; Dosdall and Lehmkul, 1989; Sibley *et al*., 1991). *Gammarus pulex* drift during runoff contaminated with insecticides was significantly increased compared to runoff without insecticide contamination (Liess *et al*., 1993). Several studies have documented that BMI drift is a significant determinant of BMI community dynamics (Dermott and Spence, 1984; Liess *et al*., 1993; Taylor *et al*., 1994).

Schulz *et al*. (2002) evaluated the potential aquatic ecosystem effects of the OP insecticide azinphosmethyl in a combined microcosm and quantitative macroinvertebrate bioassessment investigation. The focus of the study was the Lourens River in South Africa. The upper regions of the river are free of contaminants (reference sites located in this section), whereas subsequent stretches of the river flow through orchard areas that receive transient OP insecticide input. The BMI bioassessment was performed after the seasonal azinphosmethyl application to the orchards. Their results provided robust indications that transient OP insecticide contamination impacts on aquatic community integrity in the Lourens River.

Leonard *et al*. (1999, 2001) investigated invertebrate species at eight sights on the Namoi River (southeastern Australia) in relation to endosulfan runoff from cotton fields. River invertebrate species were clearly impacted by the runoff. Study results linked dynamics of the six dominant species with insecticide contamination. Two streams contaminated by endosulfan runoff and one uncontaminated stream in Argentina were investigated by Jergentz *et al*. (2004). Benthic macroinvertebrate dynamics and drift were impacted in the two endosulfan-contaminated streams compared to the uncontaminated stream.

Hatakeyama and Yokoyama (1997) explored the potential effects of rice field runoff on the Suna River in Japan. Benthic macroinvertebrate surveys and ambient water toxicity tests with an indigenous shrimp were applied to assess potential effects of the runoff. These two procedures were applied upstream and downstream of rice field inputs after the application of pesticides to those fields. Benthic macroinvertebrate community integrity below the input of rice fields was impacted compared to the upstream sites. Ambient water tests with the shrimp also revealed that the runoff from the rice fields were toxic compared to the upstream sites. Moreover, the laboratory toxicity tests 'predicted' the impacts to the benthic macroinvertebrates. While there was some recovery of the benthic macroinvertebrate community from fall to spring, recovery was incomplete. Runoff from rice fields also was shown to impact benthic macroinvertebrate community integrity in another river in Japan (Tada and Shiraishi, 1994). These authors concluded that rice pesticides were the cause of the impacts, but there was no confirmation of this hypothesis. Nonetheless, some component(s) of the runoff were responsible for the impacts. References to these studies is not intended to imply that an identical situation occurs in the California Central Valley, because practices related to discharge of rice irrigation water differs from those in Japan.

Several other studies provide evidence of agriculture-derived insecticide impacts on stream or river water quality and BMI community integrity (Heckman, 1981; Baughman *et al*., 1989; Sallenave and Day, 1991; Liess *et al*., 1993; Lenat and Crawford, 1994; Matthiesen *et al*., 1995; Liess and Schulz, 1996; Schulz and Liess, 1997: Liess and Schulz, 1999; Schulz, 2004). Cuffney *et al*. (1984) documented that a pyrethroid insecticide contamination of an aquatic ecosystem not only altered BMI community integrity, but also ecosystem processes. Pesticides and other agricultural chemicals have been implicated in a host of sub-lethal effects on aquatic species including endocrine disruption, immunosupression (susceptibility to pathogens and disease), embryonic development and growth, salmonid olfactory function (impairing migratory and spawning abilities), and behavioral abnormalities (including inhibition of predator avoidance and feeding success).

Schulz (2004) reviewed studies published since 1982 related to insecticide (originating from agricultural runoff or spray drift) occurrence in surface waters and effects on aquatic ecosystem biota. With regard to the effects of agriculture-derived insecticides on aquatic biota, he categorized the studies reviewed into three categories: (1) Study assumed a relationship [Evidence pointed to impacts of insecticide(s), but there were no chemical quantifications], (2) Study provided evidence of a likely relationship and (3) Study yielded clear evidence of a relationship. Schulz classified 16, 5 and 21 published studies reviewed into categories 1 through 3, respectively. One approach to assess insecticide-caused water quality degradation is comparison of water quality standards (or criteria, guidelines) to surface water concentrations of the insecticide. According to Schulz (2004) insecticides with the largest number of exceedances of such benchmarks are endosulfan (14 studies), chlorpyrifos (11 studies), diazinon (11 studies) and azinphos-methyl (9 studies). We concur with his prediction that exceedances would have been much higher if sampling would have been event-based rather than random or on a fixed temporal schedule. There are limitations to this comparison approach. Limitations include (1) Chemical measurements of a constituent in surface water or sediment do not necessarily indicate bioavailability (and, therefore, toxicity) of that substance to biota and (2) Approach does not consider additive, synergistic, cumulative, or antagonistic interactions with other contaminants, water quality parameters, physical/habitat stressors.

Insecticide runoff into waterways is frequently temporally variable, commonly a pulsed phenomenon. Thus, monitoring programs/projects should include a component of event-based (e.g., following insecticide applications and with subsequent irrigation; after storms) sampling. This phenomenon is particularly true of the smaller drains. While there are several studies that have explored such ‘pulsed flow/input’, further investigation into the biological/ecological effects and recovery are needed.

Agricultural drains and agriculture-dominated waterways in California's Central Valley have been contaminated by sediment, pesticides, other agricultural chemicals, metals, organic wastes, nutrients, salts and organic carbon originating from agricultural activities for 40 or more years. Thus, it is likely that most aquatic biota occurring in these waterways are tolerant to these stressors. A recent bioassessment study conducted by UCD ATL supports this hypothesis (de Vlaming *et al*., 2004b). In a recent review article Schulz (2004) suggested that 'ghosts of disturbances past' are likely to cause difficulty in detecting pesticide-related effects on existing communities because pesticide effects were exerted years ago. The question to be debated is whether to attempt to maintain 'best existing biological' conditions or strive to restore improved biological conditions.

4.5 Monitoring Data Interpretation

Interpretation and evaluation of monitoring data could be facilitated, as well as more objective and equivalent, if science-based aquatic ecosystem standards, including biological criteria, were in place. Toxicological and chemical standards should include magnitude, duration, frequency and geographic extent components. Development of effective standards depends on accurate designation of beneficial uses. Aquatic life beneficial uses are likely to vary with order (e.g., ranking based on size and flow) of drain or other agriculture-dominated waterway. Bioassessment work performed at UCD ATL sustains this suggestion and also indicated that BMI community structure tended to 'cluster' (be more similar) by waterway and watershed (de Vlaming *et al*., 2004b). Thus, consideration should be given to designation of agricultural drain and agriculture-dominated waterway beneficial uses on a tiered/stratified basis. Aquatic ecosystem standards (criteria, guidelines) would follow the same tiering.

5. Recommendations

The following recommendations do not deal with all aspects and components of water quality monitoring programs or projects. For a more complete coverage of designing water quality programs, see Maher and Batley (2002). These recommendations relate to follow-up on data generated in this project, to experiences encountered in this project, and to information gained in the literature review.

- A program and plan for monitoring water quality and biological condition in agricultural drains and ADWs is needed. We recommend a consistent, long-term and widespread monitoring and assessment program that focuses on these systems.
- To enhance potential for success of a monitoring program or project and ensure that data generated are reliable and credible (1) a careful and clear definition of specific objective(s), (2) a definition of intended use of data and, thus, data requirements, and (3) careful planning are critical. Planning should include a search for related existing or past projects and data, literature reviews and analyses, considerable contemplation and integration. These activities prevent unnecessary duplication of effort and contribute significantly to study design. These activities are effort and time intensive. Care should be taken that program and project budgets allot adequate funds for these activities.
- Quality assurance is an essential component of monitoring programs and projects. However, uncertainty in science is a reality that cannot be totally eliminated (National Research Council, 2001). To avoid consumption of large sums of monitoring budgets, we advise defining a priori the level of uncertainty that is acceptable.
- Site selection and reconnaissance are critical to successful monitoring projects. Such efforts are labor and time intensive, so project budgets should designate sufficient funds for these efforts.
- If a project goal is to ascertain whether agricultural runoff is causing water quality degradation and impacts on aquatic ecosystem biota, neither site selection nor sampling timing should be random (probabilistic based). Site selection should be associated with inputs of agricultural runoff/discharges and sampling should be event-based (e.g., considering irrigation regimes, other hydrological activities, use of pesticides and other agricultural chemicals, storm runoff, etc.), associated with agricultural practices most likely to impact water quality and aquatic ecosystem biota.
- Pulses of contaminants are common in agricultural drains and ADWs. Thus, as indicated above, we recommend that monitoring projects include high frequency event-based sampling. Luoma *et al*., (2001) concluded that a combination of spatially extensive and

temporally intensive sampling designs is necessary to understand the influence of multiple stressors on aquatic ecosystems. We also advise that potential ecological effects of such types of pulse exposures be further investigated.

- Water environments are naturally variable. Design of monitoring projects should be cognizant of this variability. Further, defining *natural* temporal and spatial variation in aquatic systems is vital to interpretation of monitoring data, especially bioassessment data.
- Proposing a specific water quality monitoring design that would 'fit' all agricultural drains and ADWs is challenging because of the host of variables to consider. From our perspective important variables to consider in the design of a specific monitoring project include objective(s); project budget; intended use of data; existence or absence of previous monitoring data; cropping patterns; irrigation patterns, frequency, volume, flow and velocity; quantities, timing, and frequencies of agricultural chemical applications; and size/width of waterway and proximity of sampling site to agricultural lands (i.e., location of sites along the length of the waterway).
- Collection of monitoring data alone cannot protect, improve or restore water quality or biological condition in aquatic systems. Programs should avoid being or becoming data collection exercises. Interpretive/integrative reports, solutions and actions are essential for protection and restoration of degraded waterways in California. Preparation of integrative/interpretive reports is very labor and time intensive. If such reports are desired, monitoring project budgets should include adequate funds to cover actual costs of preparation.
- Study design and data interpretation for non-point source waterway monitoring are much more complex than for point source discharges because many more variables/parameters have to be considered. Collecting data on multiple variables simultaneously is expensive. To defray expenses we recommend that non-point monitoring projects, when possible, include multiple agencies and entities so that pertinent watershed data (e.g., irrigation, hydrology, watershed and waterway geomorphology, stream and riparian habitat, and soils data) are collected.

- Consideration should be given to designation of agricultural drain and agriculture-dominated waterway beneficial uses on a tiered/stratified basis. Aquatic ecosystem regulatory standards (criteria, guidelines) should follow the same tiering.
- The Central Valley Regional Water Quality Control Board has an enforceable narrative water quality objective (standard) for toxicity and enforceable chemical specific numeric objectives for a limited range of contaminants. Interpretation and evaluation of monitoring data could, however, be facilitated if science-based aquatic ecosystem numeric standards were in place. Toxicological and chemical standards should include magnitude, duration, frequency and geographic extent components. From our perspective, such standards are needed for water column and sediment toxicity, sediment loads, turbidity/TSS, TOC, and specific conductivity in agricultural drains and ADWs.
- Because multiple stressors originating from agricultural practices are the norm, water quality standards alone will not always protect or restore aquatic biological community integrity in agricultural drains, ADWs, or any other waterways. The impact of agricultural practices on beneficial uses (biological integrity and health) can be severe because of the compounded nature of perturbations (Luoma *et al*., 2001). In this regard, monitoring programs and projects should encompass all potential stressors on biological integrity and other beneficial uses. We recommend that monitoring projects include systematic and simultaneous collection of physical, chemical, toxicological and biological data from aquatic systems in a weight-of-evidence approach. In agricultural drains and ADWs, there is a particular need to include sediment toxicity testing, TIEs and chemical analyses. Sediment toxicity testing is more likely to identify pyrethroid insecticide impacts than is water column testing.
- While ATL recommends multiple-procedure monitoring projects (unless previously collected data focus the need for a specific monitoring procedure or the particular objective or question to be answered requires the use of a particular method). We recognize that limited budgets disallow such extensive projects in many cases. When budgets are limited we propose that initial decisions should consider whether the project focus is on water quality or beneficial uses (biological condition) assessment.
- Bioassessments should be a component of agricultural drain and ADW monitoring projects. Data collected in bioassessment studies should meet quality assurance criteria that include

representativeness, completeness, comparability, precision and procedure sensitivity. For recommendations related to use of bioassessments in agriculture-dominated waterways see de Vlaming *et al*. (2004b).

- We support the development of biological standards/criteria for waterways in the Central Valley. However, biological communities in the Central Valley are poorly understood. Furthermore, there are no agricultural drains or ADWs unimpacted by human activity, so true reference sites do not exist. Without reference sites and a more complete understanding of biological communities in the Central Valley it will be difficult to define natural temporal and spatial variation in biological populations. Without reference sites and knowledge of natural temporal and spatial variation, development of biological criteria will be extremely challenging. We recommend that this issue be addressed.
- Follow-up investigations at sites where toxicity was observed in this study are recommended. Such studies should include more frequent sampling and physical, chemical, toxicological and biological monitoring procedures (i.e., weight-of-evidence approach), as well as a design that will enable assessment of geographic extent of possible effects.
- This study identified a possible relationship between drain toxicity and irrigation regimes/patterns. Follow-up investigations, with designs focused on identification of potential relationships (irrigation regime and volume with runoff water quality), are recommended.
- Recycling, rather than discharge, of irrigation runoff would likely decrease water quality degradation in ADWs.
- Strategies/management practices should be developed to reduce offsite movement of sediment, as well as chlorpyrifos, other pesticides and agricultural chemicals.
- Studies focused on potential water quality effects of irrigation runoff should, when possible, include examination of irrigation source water as a reference.

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